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Contract No.: DAMD17-92-C-2001
Task Order No.: UIC-7I
UIC/TRL Study No.: 133

Title Page

Study Report for Task Order No. UIC-7I

FOUR WEEK ORAL DOSE RANGE-FINDING STUDY OF WR242511 IN DOGS

Sponsor: US Army Medical Materiel Development Activity

Test Article: WR242511

Contract No.: DAMD17-92-C-2001

Study Director

Barry S. Levine, D.Sc., D.A.B.T.

In-Life Phase Completed On

March 9, 1994

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UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY Unclassified	3. DISTRIBUTION / AVAILABILITY OF REPORT Unlimited
2b. DECLASSIFICATION / DOWNGRADING SCHEDULE UIC-7I (UIC/TRL Study No. 133)	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION Toxicology Research Laboratory University of Illinois at Chicago 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION U.S. Army Medical Materiel Development Activity
6c. ADDRESS (City, State, and ZIP Code) Department of Pharmacology (M/C 868) 1940 W. Taylor Street Chicago, IL 60612-7353	7b. ADDRESS (City, State, and ZIP Code) ATTN: SGRD-RMA-RCD Fort Detrick Frederick, MD 21702-5014
8a. NAME OF FUNDING / SPONSORING ORGANIZATION U.S. Army Medical Materiel Development Activity 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER DAMD17-92-C-2001
8c. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, MD 21702-5009	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. 63807A; PROJECT NO. 30463807; TASK NO. QC; WORK UNIT ACCESSION NO. 073

11. TITLE (Include Security Classification)

Four Week Oral Dose Range-Finding Study of WR242511 in Dogs

12. PERSONAL AUTHOR(S)
Levine, Barry S. and Wheeler, Clyde W.

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Draft	FROM 11/19/93 TO _____		

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	WR242511 Toxicity Dogs

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

This study evaluated the toxicity of WR242511 tartrate in dogs following four weeks of daily administration by gelatin capsule. The primary toxic effects of WR242511 tartrate were seen in the RBCs, liver and the lung. Anemia and methemoglobinemia were observed in all the dose levels tested. Compensatory changes to the anemic state included macrocytosis, reticulocytosis and increased nucleated RBCs. Decreases in body weight and food consumption were seen at the high dose level and possibly the mid dose level. Microscopic lesions were seen in the liver and the lung at all dose levels tested. Hepatocellular swelling was supported by decreases in the A/G ratio and increases in haptoglobin levels in mid and/or high dose animals. Pulmonary lesions (alveolar proteinic exudates, macrophage infiltration and acute alveolar inflammation) and mild to moderate thrombocytopenia were seen at all dose levels. Leukocytosis consisting of increased mature neutrophils, possibly secondary to stress, was seen in high animals and possibly in the mid dose female. On the basis of the findings from this study and after consultation with the Sponsor, the following three dose levels have been selected for use in the four week oral toxicity study: 0.1, 0.3 and 1.0 mg base/kg/day.

20. DISTRIBUTION / AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION	
☐ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	Unclassified	
22a. NAME OF RESPONSIBLE INDIVIDUAL Barry S. Levine	22b. TELEPHONE (Include Area Code) (312) 996-5543	22c. OFFICE SYMBOL N/A

DD Form 1473, JUN 86 *Previous editions are obsolete.*

SECURITY CLASSIFICATION OF THIS PAGE

UNCLASSIFIED

Contract No.: DAMD17-92-C-2001
Task Order No.: UIC-7I
UIC/TRL Study No.: 133

Signature Page

FOUR WEEK ORAL DOSE RANGE-FINDING STUDY OF WR242511 IN DOGS

Test Article: WR242511

Sponsor: US Army Medical Materiel
Development Activity
Fort Detrick
Frederick, MD 21702-5014

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9/22/94
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9/22/94
Date

Study Initiation: November 19, 1993
Dosing Initiation: February 9, 1994
In-Life Completion: March 9, 1994

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1. SUMMARY

This study evaluated the toxicity of WR242511 tartrate in dogs following four weeks of daily administration by gelatin capsule. The results are summarized in Table 1. The primary toxic effects of WR242511 tartrate were seen in the RBCs, liver and the lung. Anemia and methemoglobinemia were observed in all the dose levels tested. Compensatory changes to the anemic state included macrocytosis, reticulocytosis and increased nucleated RBCs. Decreases in body weight and food consumption were seen at the high dose level and possibly the mid dose level. Microscopic lesions were seen in the liver and the lung at all dose levels tested. Hepatocellular swelling was supported by decreases in the A/G ratio and increases in haptoglobin levels in mid and/or high dose animals. Pulmonary lesions (alveolar proteinic exudates, macrophage infiltration and acute alveolar inflammation) and mild to moderate thrombocytopenia were seen at all dose levels. Leukocytosis consisting of increased mature neutrophils, possibly secondary to stress, was seen in high animals and possibly in the mid dose female. On the basis of the findings from this study and after consultation with the Sponsor, the following three dose levels have been selected for use in the four week oral toxicity study: 0.1, 0.3 and 1.0 mg base/kg/day.

2. INTRODUCTION

This study was conducted to determine the appropriate dose levels for a four week oral toxicity study of WR242511 tartrate by gelatin capsule. The study was conducted in accordance with the specifications of the Sponsor, as indicated in Task Order UIC-7I. The FDA requires the use of two animal species, one of which is a non-rodent, in preclinical toxicology studies. The dog is a standard and accepted non-rodent species for regulatory toxicology studies, and was specified by the Sponsor. Oral administration is the intended clinical route and was also specified by the Sponsor. All methods and procedures were conducted within the spirit of the Quality Assurance Programs of the Toxicology Research Laboratory, University of Illinois at Chicago and Pathology Associates, Inc. designed to conform with FDA Good Laboratory Practices Regulations. No unforeseen circumstances affected the integrity of the study. Dosing was initiated on February 9, 1994 and the in-life portion was terminated on March 9, 1994.

3. MATERIALS AND METHODS

3.1 Test Article

WR242511 Tartrate (Lot No. DJD-08-235, Batch No. BM05816) a yellow powder, was received on June 16, 1993 from Herner & Co. and was assigned an in-house chemical number (1720614). The chemical name of the test article is 8-[(4-Amino-1-methylbutyl)amino]5-(1-hexyloxy)-6-methoxy-4-methylquinoline DL tartrate and the mole fraction of the base is 0.71. It was stored at -20 to -15°C, ambient humidity and protected from light in an amber bottle.

The test article purity had previously been determined (1/24/94) at the completion of the in-life portion of a study entitled "Thirteen Week Oral Toxicity Study of WR242511 in Rats" (UIC/TRL Study No. 107). At that time, the purity was 99.59% $\pm$ 0.02%.

3.2 Animals

A shipment of male and female Beagle dogs were obtained from Marshall Farms, North Rose, NY on November 18, 1993. The animals were approximately 6 - 7 months old (birth dates between April 16, 1993 and May 15, 1993) upon arrival at the UIC AAALAC-accredited animal facility. Each animal was given a facility-unique animal number upon arrival. This number immediately appeared as a tag on a chain collar, and was additionally tattooed on the inner aspect of the ear on the same day. Animals were singly housed, except as noted, in runs in a temperature (72 $\pm$ 6°F) and humidity (50 $\pm$ 20%) controlled room with a 12 hour light/12 hour dark cycle. During the quarantine/pretest period, the animals were occasionally housed two/run within sex. The run size, typically at least 15 square feet, was adequate to house dogs at the upper weight range as described in the *Guide for the Care and Use of Laboratory Animals*, DHHS (NIH) No. 86.23. All runs were cleaned and fresh bedding was replaced daily. The runs were sanitized once every two weeks.

Certified Canine Diet No. 5007 (PMI Feeds Inc., St. Louis, MO), approximately 400 g, was provided daily from arrival until termination. Exactly 400 g were provided when food consumption was measured. The food was removed for an overnight fast ($\approx$ 16 - 20 hours) prior to blood collection and scheduled sacrifice. Tap water was provided *ad libitum* from an automatic watering system in which the room distribution lines were flushed daily from arrival until termination. The water was untreated with additional chlorine or HCl. There were no known contaminants in the feed or water which were expected to influence the study. The results of the most current comprehensive chemical analyses of Chicago water are documented in files maintained by Quality Assurance.

Animals were quarantined for approximately two months, as they were selected from a shipment used for several studies. Body weights and physical examinations were done upon the dogs' arrival at the animal facility. Additionally, each dog was lightly sprayed upon arrival with Para Pyrethrin Mist for fleas, lice, and ticks. At least one week prior to dosing initiation, hematology and clinical chemistry tests, and fecal examination for internal parasites were performed. All dogs had been previously vaccinated against canine distemper, infectious canine hepatitis, leptospirosis, parainfluenza, parvo, oral papilloma, and rabies by the animal supplier. For approximately three weeks prior to dosing initiation, the animals were observed daily for signs of illness and all unusual observations were reported to the Study Director, Toxicologist, or Clinical Veterinarian. Animals were examined during quarantine and approved for use by the Clinical Veterinarian prior to being placed on test. Quarantine release was documented on the Clinical Veterinarian Log by the veterinarian prior to study initiation.

3.3 Experimental Design

Three animals of each sex were chosen from the shipment on the basis of quarantine data including body weight, food consumption and clinical pathology. These animals were randomized by sex using a table of random letters into the groups shown in the following table.

Treatment Group	Dose Level (mg base/kg/day)	Number of Males	Number of Females
1	0.5	1	1
2	0.9	1	1
3	1.5	1	1

WR242511 dose levels were selected on the basis of a previously conducted two week oral dose range-finding study in rats (UIC/TRL Study No. 106), following consultation with the Sponsor. The number of animals, 1 sex/dose, is the minimum number of dogs and is the number typically used in preliminary dose range-finding studies.

Following treatment group allocation, the animal's number appeared on a card visible on the front of each run. The run card additionally contained the study number, test article identification, treatment group number, sex and dose level. Run cards were color-coded as a function of treatment group.

The test article was administered once daily by gelatin capsule starting with Day 0 (February 9, 1994) for four weeks. All animals received empty gelatin capsules for the last 3 days during Week -1 to acclimate them to the procedure. The quantity of the test article (mg base/kg/day) was adjusted based on the animal's most recent body weight. The animals were dosed up to and including the day prior to scheduled necropsy on Day 28. The dogs weighed 9.7 - 10.7 kg (males) and 8.4 - 9.1 kg (females) on Day -2, and were approximately 9 - 10 months old at initiation of treatment.

Body weights of all animals were recorded at randomization in Week -1, weekly thereafter, and at termination on Day 28. Clinical signs were recorded once daily, approximately 1 - 2 hours after dosing. The general behavior, posture, locomotion, breathing pattern and coat were observed for all animals. The animals were also observed immediately prior to dosing and in the afternoon for moribundity/mortality. Physical examinations (clinical observations) which included examination of eyes and all orifices were conducted in Week -1, on Day 0 prior to dosing, and once weekly thereafter. Food consumption was measured for all animals over an approximate 24 hour period once weekly commencing with Week -1.

Hematology and clinical chemistry parameters were measured following an overnight fast approximately one week prior to dosing initiation, on Day 14 and on Day 28 at termination. In addition, overnight fasted methemoglobin levels were measured weekly commencing on Day 0, just prior to dosing. On Day 21, in addition to the measurement of methemoglobin levels prior to dosing, non-fasted levels were determined approximately 2 and 8 hours after treatment. The animals were unanesthetized and sufficient blood was collected from the jugular vein to measure the following parameters in random order. Water was available *ad libitum* during all fasting periods. Clinical pathology methodology is contained in Appendix 1.

Hematology

Activated partial thromboplastin time	Mean corpuscular hemoglobin (MCH)
Erythrocyte count	Mean corpuscular hemoglobin concentration (MCHC)
Erythrocyte morphology	Mean corpuscular volume (MCV)
Heinz bodies	[a]Methemoglobin
Hematocrit	Platelet count
Hemoglobin	Prothrombin time
Leukocyte count, total and differential	Reticulocyte count

[a]Measured with a Co-oximeter (Instrumentation Laboratory, Model No. 282). The assay was performed within one-hour of sample collection. The specimens were kept on wet ice prior to analysis.

Clinical Chemistry

Alanine aminotransferase (ALT/SGPT)	Globulin (calculated)
Albumin	Glucose
Albumin/globulin ratio (calculated)	Haptoglobin
Alkaline phosphatase	Lactate dehydrogenase (LDH)
Aspartate aminotransferase (AST/SGOT)	Phosphorus (inorganic)
Calcium	Potassium
Chloride	Sodium
Cholesterol	Total bilirubin
Creatinine	Total protein
Creatine kinase (CK)	Triglycerides
Gamma glutamyl transferase	Urea nitrogen (BUN)

Additionally, a minimum of 2.5 ml of blood was collected from the jugular vein weekly, just prior to dosing, beginning on Day 0 for the separation and isolation of plasma and cellular blood components according to the Sponsor's directives. The plasma and cell fractions resulting from separation by centrifugation were sent to COL Thomas Brewer, WRAIR, as specified by the Sponsor. The results obtained from these samples are not included in the study report.

All animals survived the four week treatment period and were sacrificed and necropsied on Day 28. This was accomplished by sodium pentobarbital anesthesia (i.v.; 20-30 mg/kg) and exsanguination. An extensive necropsy was performed under the direction and supervision of the pathologist. Terminal body weights were collected prior to routine sacrifice.

The necropsy procedure was a thorough and systematic examination and dissection of the animal viscera and carcass to include the external surface, all orifices, the cranial cavity, external surface of the brain, cross section of the spinal cord, the nasal cavity and nasal turbinates, thoracic, abdominal and pelvic cavities and their viscera, and cervical tissues and organs. The following tissues and organs were collected and fixed in 10% neutral buffered formalin (NBF).

*Adrenal glands
Aorta
*Brain (fore-, mid-, and hind-)
Cecum
Colon
Diaphragm
Duodenum
Epididymides
Esophagus
Eyes and optic nerve
Gall bladder
Gross lesions
*Heart
Ileum
Jejunum
*Kidneys
*Liver (with gall bladder drained)
*Lungs/Bronchi
Lymph node (submandibular)
Lymph node (mesenteric)
Mammary gland
Muscle (skeletal)
Nerve (sciatic)
*Ovaries
Pancreas
Pituitary
Prostate
Rectum
Rib with marrow
Salivary gland (submandibular)
Skin
Spinal cord (thoracic, cervical)
*Spleen
Stomach
*Testes
Thymus
*Thyroid gland with parathyroids
Tongue
Tonsil
Trachea
Ureter
Urinary bladder
Uterus

Those tissues marked with an asterisk (*) were embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically in all animals necropsied on Day 28.

In addition to the collection of the aforementioned tissues and organs, five tubes of heparinized blood (≈ 250 ml) and bile samples aspirated by syringe from the gall bladder were collected at necropsy according to the Sponsor's directives. These samples were sent to COL Thomas G. Brewer, WRAIR, as specified by the Sponsor, and the results obtained from these samples are not included in the study report.

3.4 Statistical Analysis

Statistical analyses were not conducted due to the small sample size. The dose levels for all the summary and individual data are expressed on the basis of mg base/kg/day.

Quantitative data were tabulated and presented in the report. In addition to the written report, summary data tables of parameters and variability were transmitted to the Sponsor on magnetic media (computer diskette) in "ASCII" form.

RESULTS

4.1 Mortality/Clinical Signs

Summary of clinical signs are presented in Table 2. Individual clinical signs and daily incidence of clinical signs are contained in Appendix 2.

No animals died during the study. Treatment-related daily clinical signs of cyanosis (1 - 2 hours post-dosing) were observed in all treatment groups. Signs of cyanosis were first observed beginning on Days 1 and 2 in the two high dose animals and beginning on Days 6 and 7 in the low dose animals. The severity increased from slight (barely perceptible, slight blue tinged color) blue gums and sclera and moderate (easily seen blue color) blue tongue in low dose animals to moderate blue gums and severe (marked, deep blue-purple color) tongue in mid and high dose females. The severity of these cyanotic signs appeared to reach a plateau in the second week of treatment and were thereafter observed for the remainder of the study. A few exceptions existed including an increase in severity of signs of cyanosis in the mid and high dose female in Week 4 and the disappearance of severe blue tongue in these same animals after Day 21. Pale gums and tongue (lacking a pink appearance) were also occasionally observed in all dose levels, but was frequently noted for the high dose male. Diarrhea was seen once in the low dose female and emesis was seen several times in the low dose male.

4.2 Body Weight

Individual body weights and individual weight gains are presented in Tables 3 and 4, respectively.

During the treatment period, both high dose animals lost 0.5 kg. Body weight changes in the low and mid dose animals during this period were marginal, ranging from 0.1 to -0.2 kg.

4.3 Food Consumption

Individual food consumption data are shown in Table 5.

Food consumption appeared to be occasionally decreased in mid and/or high dose animals. Food intake did not appear to be altered in low dose animals.

4.4 Clinical Pathology

Individual animal clinical chemistry data are shown in Tables 6.1 - 6.6. Individual animal hematology data are shown in Tables 7.1 - 7.6. Individual animal methemoglobin data are presented in Tables 8.1 and 8.2.

Apparent decreases in serum albumin levels and/or increases in serum globulin levels resulted in a decreased A/G ratio in mid and high dose animals. These changes suggest that WR242511 possibly produced marginal hepatotoxic changes.

Serum haptoglobin levels were below the detection limit (< 13 mg/dl) in all females on Day -7/-8 and the low dose female on Day 14. On Day 14, increases in serum haptoglobin levels were seen in mid and high dose animals, and the low dose male. On Day 28, all animals had increased serum haptoglobin levels. The occurrence of increased levels of this protein, which is synthesized by hepatocytes, is indicative of an inflammatory response, i.e. an acute phase reaction.

Dose-dependent anemia, as indicated by decreased RBC count, hemoglobin and hematocrit, were seen in mid and high dose animals and in the low dose male. The maximal effect was generally seen after two weeks of treatment (Day 14), and some resolution of the anemia was observed by Day 28 in the two higher dose levels. In contrast, the apparent anemia in the low dose male was not observed until Day 28. Compensatory increases in MCV, reticulocyte counts and/or nucleated RBCs were seen in mid and high dose animals and in the low dose male.

Mild to moderate thrombocytopenia was seen in all animals, except in the low dose female. The greatest decrease in platelet count was seen on Day 14. By Day 28, the thrombocytopenia had started to resolve. Slight increases in WBC counts were observed in high dose animals. This leukocytosis was apparently a result of increased mature neutrophil numbers.

Biologically significant elevations of methemoglobin levels were seen in all dose levels. These levels appeared to peak by Day 14 and slightly decrease thereafter. By comparing methemoglobin levels at 2 and 8 hours post dosing (Day21-2h and Day21-8h, respectively) with those measured prior to dosing on Day 21 (Day21-0h), it appeared that methemoglobin levels were at a steady-state level.

4.5 Pathology

The Pathology Report is contained in Appendix 3. A summary of microscopic lesions is shown in Table 9.

The oral administration of WR242511 was associated with changes in the lung and the liver. The lung lesions (alveolar proteinic exudates, macrophage infiltration and acute alveolar inflammation) were observed in the high dose animals, in the mid dose female, and in the low dose male. Proteinic exudate which consisted of amphorous to fibrillar gray-pink acellular material was observed in the alveolar lumen. Macrophage infiltrates were observed mainly in the interstitial tissues near terminal bronchioles, but also were present in the interstitium and in alveoli. These infiltrating macrophages were large cells with abundant, pale, vacuolated cytoplasm. Acute alveolar inflammation was characterized by neutrophil infiltrations with necrosis.

Swollen hepatocytes, a common morphologic manifestation of degenerative changes, was seen in all animals, except in the low dose male. This change was identified as large cells whose cytoplasm had a ground-glass appearance.

No other microscopic changes were considered to be related to WR242511 treatment.

5. DISCUSSION

This study evaluated the toxicity of WR242511 tartrate in dogs following four weeks of daily administration by gelatin capsule. The results are summarized in Table 1. No animals died during the study. Generalized cyanosis manifested clinically by blue gums, sclera and tongue was observed in all dose levels and was supported by methemoglobinemia. In addition, pale gums and tongue was observed in most animals. An increase in severity and duration was observed in the higher dose levels especially in the females. Decreases in body weights accompanied by sporadic decreases in food consumption were seen in the high dose animals and possibly in the mid dose female.

Treatment-related anemia, as indicated by decreased RBC, hemoglobin and hematocrit, was apparent in all dose levels, but not in the low dose female. Macrocytosis, reticulocytosis and an increase in nucleated RBCs were seen as compensatory responses to the anemic state in mid and high dose animals. Methemoglobinemia was seen in all dose levels throughout the study. Methemoglobin appeared to be maintained at steady state levels by Day 21.

WR242511-induced hepatocyte swelling was noted in all dose levels tested. This lesion was of minimal severity in the affected animals. The hepatocellular swelling may have been associated with the apparent decreases in the A/G ratio, and increases in serum haptoglobin levels, indicative of an acute phase reaction.

In the lung, WR242511 resulted in alveolar proteinic exudates, macrophage infiltration and acute alveolar inflammation. These lesions of minimal to mild severity were observed in the high dose animals, in the mid dose female, and in the low dose male.

Mild to moderate thrombocytopenia was seen at all dose levels. Leukocytosis consisting of increased mature neutrophils was seen in high dose animals. The neutrophilia was possibly an indirect effect of the stress produced by the anemia and methemoglobinemia.

The purpose of this study was to select dose levels for a four week oral toxicity study of WR242511 in dogs. It is anticipated that frank toxicity would occur at the high dose level, marginal or no toxicity accompanied by potentially therapeutic methemoglobin levels would be seen at the mid dose level, and no toxicity accompanied by minimal elevation in methemoglobin levels would be observed at the low dose level. On this basis and after consultation with the Sponsor, the following three dose levels have been selected for use in the four week oral toxicity study: 0.1, 0.3 and 1.0 mg base/kg/day.

6. PERSONNEL

Study Director	Barry S. Levine, D.Sc., D.A.B.T.
Toxicologist	Clyde W. Wheeler, Ph.D.
Pathologist	Michael J. Tomlinson, D.V.M., Ph.D., D.A.C.V.P.
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Clinical Veterinarian	Terry Hewett, D.V.M., D.A.V.C.P.
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Chemistry Specialist	Thomas Tolhurst, B.S.
Clinical Pathology	Maria Lang, A.H.T., C.V.T.

Report preparation was assisted by Rae-Jean T. Ballentine, B.S.

7. ARCHIVES

The raw data, specimens, test article reserves, and final report are archived at the Toxicology Research Laboratory (TRL), University of Illinois at Chicago (UIC), Department of Pharmacology, 1940 W. Taylor St., Chicago, IL 60612-7353.

Table 1

FOUR WEEK ORAL DOSE RANGE-FINDING
STUDY OF WR242511 IN DOGS

Summary of Toxic Responses

Dose (mg base/kg/day)	0.5	0.9	1.5
Dogs/Sex	●	●	●
Deaths	0	0	0
Body Weight Gain	NE	↓ F?	↓
Food Consumption	NE	↓ F?	●
Clinical Signs	Blue gums Blue sclera Blue tongue Pale gums (F) Pale tongue (F)	Blue gums Blue sclera Blue tongue Pale gums Pale tongue (M)	Blue gums Blue sclera Blue tongue Pale gums Pale tongue (M)
Clinical Chemistry[a]	↑ HPT	↓ ALB ↑ GLOB ↓ A/G ↑ HPT	↓ ALB ↑ GLOB ↓ A/G ↑ HPT
Hematology[b]	↓ RBC (M) ↓ HGB (M) ↓ HCT (M) ↑ MCV (M) ↑ METHB ↓ PLT (M)	↓ RBC ↓ HGB ↓ HCT ↑ MCV ↑ RETICS ↑ NRBCs ↑ METHB ↓ PLT	↓ RBC ↓ HGB ↓ HCT ↑ MCV ↑ RETICS ↑ NRBCs ↑ METHB ↓ PLT ↑ LEUK ↑ MNEUT
Histopathology	LUNG - Alveolar proteinic exudate (M) Macrophage infiltration (M) Acute alveolar inflammation (M) LIVER - Hepatocellular swelling (F)	LUNG - Alveolar proteinic exudate (F) Macrophage infiltration (F) Acute alveolar inflammation (F) LIVER - Hepatocellular swelling	LUNG - Alveolar proteinic exudate Macrophage infiltration Acute alveolar inflammation LIVER - Hepatocellular swelling
Conclusions	The primary toxic effects of WR242511 tartrate were seen in the RBCs, liver and the lung. Anemia and methemoglobinemia were observed in all dose levels tested. Compensatory changes to the anemic state included macrocytosis, reticulocytosis and increased nucleated RBCs. Decreases in body weight and food consumption were seen at the high dose level and possibly the mid dose level. Microscopic lesions were seen in the liver and the lung at all dose levels tested. Hepatocellular swelling was supported by decreases in the A/G ratio, and increases in haptoglobin levels in mid and/or high dose animals. Pulmonary lesions (alveolar proteinic exudates, macrophage infiltration and acute alveolar inflammation) and mild to moderate thrombocytopenia were seen in all dose levels. Leukocytosis consisting of increased mature neutrophils, possibly secondary to stress, was seen in high dose animals and possibly in the mid dose female. On the basis of the findings from this study and after consultation with the Sponsor, the following three dose levels have been selected for use in the four week oral toxicity study: 0.1, 0.3 and 1.0 mg base/kg/day.		

[a]ALB = albumin, GLOB = globulin, A/G = albumin/globulin ratio, HPT = haptoglobin

[b]RBC = red blood cell count, HGB = hemoglobin, HCT = hematocrit, MCV = mean corpuscular volume, RETICS = reticulocyte count, NRBCs = nucleated red blood cells, METHB = methemoglobin, PLT = platelets, LEUK = leukocytes, MNEUT = mature neutrophils

? = Possible or marginal effect

NE = No effect

Table 2

FOUR WEEK ORAL DOSE RANGE-FINDING STUDY OF WR24511 IN DOGS

SUMMARY OF CLINICAL SIGNS

STUDY: 133 SEX: MALE

DOSE:(mg/kg)	0.5	0.9	1.5	(mg base/kg/day)
GROUP:	1-M	2-M	3-M	
Scheduled Sacrifice	1	1	1	
Blue Gums	1	1	1	
Blue Sclera	1	1	1	
Blue Tongue	1	1	1	
Pale Gums	0	1	1	
Pale Tongue	0	1	1	
Total Number of Animals	1	1	1	

STUDY: 133 SEX: FEMALE

DOSE:(mg/kg)	0.5	0.9	1.5	(mg base/kg/day)
GROUP:	1-F	2-F	3-F	
Scheduled Sacrifice	1	1	1	
Blue Gums	1	1	1	
Blue Sclera	1	1	1	
Blue Tongue	1	1	1	
Pale Gums	1	1	1	
Pale Tongue	1	0	0	
Total Number of Animals	1	1	1	

Table 3.1

FOUR WEEK ORAL DOSE RANGE-FINDING STUDY OF WR24511 IN DOGS

SUMMARY OF BODY WEIGHTS (Kilograms)

STUDY: 133 SEX: MALE

PERIOD	DOSE: (mg/kg) GROUP:	0.5 1-M	0.9 2-M	1.5 3-M
DAY -7	MEAN	10.7	10.6	10.1
	S.D.	0.00	0.00	0.00
	N	1	1	1
DAY -2	MEAN	10.4	10.7	9.7
	S.D.	0.00	0.00	0.00
	N	1	1	1
DAY 5	MEAN	10.3	10.5	9.4
	S.D.	0.00	0.00	0.00
	N	1	1	1
DAY 12	MEAN	10.6	10.8	8.9
	S.D.	0.00	0.00	0.00
	N	1	1	1
DAY 19	MEAN	10.3	10.5	9.0
	S.D.	0.00	0.00	0.00
	N	1	1	1
DAY 26	MEAN	10.5	10.6	9.2
	S.D.	0.00	0.00	0.00
	N	1	1	1

(mg base/kg/day)

Table 3.2

FOUR WEEK ORAL DOSE RANGE-FINDING STUDY OF WR24511 IN DOGS

SUMMARY OF BODY WEIGHTS (Kilograms)

STUDY: 133 SEX: FEMALE

PERIOD	DOSE: (mg/kg) GROUP:	0.5 1-F	0.9 2-F	1.5 3-F	(mg base/kg/day)
DAY -7	MEAN	9.4	8.5	9.2	
	S.D.	0.00	0.00	0.00	
	N	1	1	1	
DAY -2	MEAN	9.1	8.4	9.0	
	S.D.	0.00	0.00	0.00	
	N	1	1	1	
DAY 5	MEAN	8.6	8.1	8.6	
	S.D.	0.00	0.00	0.00	
	N	1	1	1	
DAY 12	MEAN	8.9	8.1	8.5	
	S.D.	0.00	0.00	0.00	
	N	1	1	1	
DAY 19	MEAN	9.2	8.2	8.2	
	S.D.	0.00	0.00	0.00	
	N	1	1	1	
DAY 26	MEAN	9.0	8.2	8.5	
	S.D.	0.00	0.00	0.00	
	N	1	1	1	

Table 4.1

FOUR WEEK ORAL DOSE RANGE-FINDING STUDY OF WR24511 IN DOGS

SUMMARY OF WEIGHT GAINS (Kilograms)

STUDY: 133 SEX: MALE

PERIOD[a]	DOSE: (mg/kg) GROUP:	0.5 1-M	0.9 2-M	1.5 3-M
DAY 5[b]	MEAN	-0.1	-0.2	-0.3
	S.D.	0.00	0.00	0.00
	N	1	1	1
DAY 12	MEAN	0.3	0.3	-0.5
	S.D.	0.00	0.00	0.00
	N	1	1	1
DAY 19	MEAN	-0.3	-0.3	0.1
	S.D.	0.00	0.00	0.00
	N	1	1	1
DAY 26	MEAN	0.2	0.1	0.2
	S.D.	0.00	0.00	0.00
	N	1	1	1
TOTAL GAIN	MEAN	0.1	-0.1	-0.5
	S.D.	0.00	0.00	0.00
	N	1	1	1

(mg base/kg/day)

[a] Successive periods

[b] Baseline is Day -2

Table 4.2

FOUR WEEK ORAL DOSE RANGE-FINDING
STUDY OF WR24511 IN DOGS

SUMMARY OF WEIGHT GAINS (Kilograms)

STUDY: 133 SEX: FEMALE

PERIOD[a]	DOSE: (mg/kg) GROUP:	0.5 1-F	0.9 2-F	1.5 3-F
DAY 5[b]	MEAN	-0.5	-0.3	-0.4
	S.D.	0.00	0.00	0.00
	N	1	1	1
DAY 12	MEAN	0.3	0.0	-0.1
	S.D.	0.00	0.00	0.00
	N	1	1	1
DAY 19	MEAN	0.3	0.1	-0.3
	S.D.	0.00	0.00	0.00
	N	1	1	1
DAY 26	MEAN	-0.2	0.0	0.3
	S.D.	0.00	0.00	0.00
	N	1	1	1
TOTAL GAIN	MEAN	-0.1	-0.2	-0.5
	S.D.	0.00	0.00	0.00
	N	1	1	1

(mg base/kg/day)

[a] Successive periods

[b] Baseline is Day -2

Table 5.1

FOUR WEEK ORAL DOSE RANGE-FINDING STUDY OF WR24511 IN DOGS

SUMMARY OF DAILY MEAN FOOD CONSUMPTION (Grams)

STUDY: 133 SEX: MALE

PERIOD	DOSE:(mg/kg) GROUP:	0.5 1-M	0.9 2-M	1.5 3-M	(mg base/kg/day)
DAY -6	INTAKE (g)	400	257	400	
	S.D.	0.0	0.0	0.0	
	N	1	1	1	
DAY -2	INTAKE (g)	347	253	400	
	S.D.	0.0	0.0	0.0	
	N	1	1	1	
DAY 6	INTAKE (g)	400	319	287	
	S.D.	0.0	0.0	0.0	
	N	1	1	1	
DAY 13	INTAKE (g)	349	286	201	
	S.D.	0.0	0.0	0.0	
	N	1	1	1	
DAY 20	INTAKE (g)	350	313	200	
	S.D.	0.0	0.0	0.0	
	N	1	1	1	
DAY 27	INTAKE (g)	321	256	400	
	S.D.	0.0	0.0	0.0	
	N	1	1	1	

Table 5.2

FOUR WEEK ORAL DOSE RANGE-FINDING
STUDY OF WR24511 IN DOGS

SUMMARY OF DAILY MEAN FOOD CONSUMPTION (Grams)

STUDY: 133 SEX: FEMALE

PERIOD	DOSE:(mg/kg) GROUP:	0.5 1-F	0.9 2-F	1.5 3-F	(mg base/kg/day)
DAY -6	INTAKE (g)	400	231	325	
	S.D.	0.0	0.0	0.0	
	N	1	1	1	
DAY -2	INTAKE (g)	355	266	261	
	S.D.	0.0	0.0	0.0	
	N	1	1	1	
DAY 6	INTAKE (g)	400	336	400	
	S.D.	0.0	0.0	0.0	
	N	1	1	1	
DAY 13	INTAKE (g)	400	206	63	
	S.D.	0.0	0.0	0.0	
	N	1	1	1	
DAY 20	INTAKE (g)	400	180	400	
	S.D.	0.0	0.0	0.0	
	N	1	1	1	
DAY 27	INTAKE (g)	331	346	400	
	S.D.	0.0	0.0	0.0	
	N	1	1	1	

Table 6.2

FOUR WEEK ORAL DOSE RANGE-FINDING
STUDY OF WR242511 IN DOGS

INDIVIDUAL ANIMAL CLINICAL CHEMISTRY DATA

STUDY ID: 133
STUDY NO: 133

GROUP: 1-F : 0.5 mg base/kg/day

SEX: FEMALE

ANIMAL ID	TEST	UNITS	DAY-7/-8	DAY 14	DAY 28
8017	ALT	U/L	37	33	36
	AST	U/L	35	48	42
	TP	g/dL	6.7	6.8	6.5
	ALB	g/dL	3.7	3.2	3.4
	GLOB	g/dL	3.0	3.6	3.1
	A/G	-	1.23	0.89	1.10
	TBILI	mg/dL	0.17	0.18	0.20
	ALKP	U/L	129	146	139
	GGT	U/L	5	3	3
	CHOL	mg/dL	214	216	195
	TRY	mg/dL	26	46	32
	LDH	U/L	101	125	42
	CK	U/L	208	183	141
	BUN	mg/dL	17.8	22.0	19.3
	CREA	mg/dL	0.87	0.93	0.84
	NA	mmol/L	147	149	148
	K	mmol/L	3.94	4.22	4.02
	CL	mEq/L	131	133	128
	CA	mg/dL	10.9	10.3	9.8
	IP	mg/dL	5.1	4.2	5.0
	GLU	mg/dL	111	125	115
	HAPT	mg/dL	--X	--X	85.2

(--)-Data Unavailable
(-)-No Units for Test

X -Below limit of Detection.

Table 6.3

FOUR WEEK ORAL DOSE RANGE-FINDING
STUDY OF WR242511 IN DOGS

INDIVIDUAL ANIMAL CLINICAL CHEMISTRY DATA

STUDY ID: 133
STUDY NO: 133

GROUP: 2-M : 0.9 mg base/kg/day

SEX: MALE

ANIMAL ID	TEST	UNITS	DAY-7/-8	DAY 14	DAY 28
7951	ALT	U/L	46	32	30
	AST	U/L	36	48	39
	TP	g/dL	6.8	6.9	6.7
	ALB	g/dL	3.7	3.1	3.1
	GLOB	g/dL	3.1	3.8	3.6
	A/G	-	1.19	0.82	0.86
	TBILI	mg/dL	0.17	0.28	0.17
	ALKP	U/L	122	64	65
	GGT	U/L	5	4	6
	CHOL	mg/dL	127	180	160
	TRY	mg/dL	28	57	40
	LDH	U/L	67	324	39
	CK	U/L	118	208	98
	BUN	mg/dL	13.7	15.0	17.1
	CREA	mg/dL	0.67	0.74	0.72
	NA	mmol/L	144	145	145
	K	mmol/L	4.81	4.74	4.54
	CL	mEq/L	126	130	QNS
	CA	mg/dL	11.1	10.4	10.0
	IP	mg/dL	6.8	4.9	4.9
	GLU	mg/dL	111	108	117
	HAPT	mg/dL	101.5	255.2	274.8

(-)-No Units for Test

QNS-Quantity Not Sufficient

FOUR WEEK ORAL DOSE RANGE-FINDING
STUDY OF WR242511 IN DOGS

INDIVIDUAL ANIMAL CLINICAL CHEMISTRY DATA

STUDY ID: 133
STUDY NO: 133
SEX: FEMALE
GROUP: 2-F : 0.9 mg base/kg/day

ANIMAL ID	TEST	UNITS	DAY-7/-8	DAY 14	DAY 28
8000	ALT	U/L	41	34	31
	AST	U/L	42	49	59
	TP	g/dL	6.7	7.0	7.1
	ALB	g/dL	3.4	3.2	2.8
	GLOB	g/dL	3.3	3.8	4.3
	A/G	-	1.03	0.84	0.65
	TBILI	mg/dL	0.27	0.27	0.18
	ALKP	U/L	74	48	73
	GGT	U/L	4	2	5
	CHOL	mg/dL	218	252	269
	TRY	mg/dL	52	74	78
	LDH	U/L	65	201	68
	CK	U/L	123	133	114
	BUN	mg/dL	15.2	18.0	15.7
	CREA	mg/dL	0.71	0.75	0.77
	NA	mmol/L	145	144	144
	K	mmol/L	4.33	4.57	4.24
	CL	mEq/L	127	132	110
	CA	mg/dL	10.6	10.4	10.1
	IP	mg/dL	5.5	5.2	5.5
	GLU	mg/dL	112	113	104
	HAPT	mg/dL	--X	97.8	42.0

(--)-Data Unavailable
(-)-No Units for Test
X -Below limit of Detection.

FOUR WEEK ORAL DOSE RANGE-FINDING STUDY OF WR242511 IN DOGS

INDIVIDUAL ANIMAL CLINICAL CHEMISTRY DATA

STUDY ID: 133
STUDY NO: 133

SEX: MALE

GROUP: 3-M : 1.5 mg base/kg/day

ANIMAL ID	TEST	UNITS	DAY-7/-8	DAY 14	DAY 28
7950	ALT	U/L	36	30	21
	AST	U/L	47	47	55
	TP	g/dL	7.3	7.7	7.2
	ALB	g/dL	3.4	2.9	2.7
	GLOB	g/dL	3.9	4.8	4.5
	A/G	-	0.87	0.60	0.60
	TBILI	mg/dL	0.15	0.18	0.12
	ALKP	U/L	99	121	113
	GGT	U/L	4	2	3
	CHOL	mg/dL	216	275	178
	TRY	mg/dL	31	69	56
	LDH	U/L	56	151	82
	CK	U/L	195	169	216
	BUN	mg/dL	21.7	13.1	16.7
	CREA	mg/dL	0.71	0.79	0.73
	NA	mmol/L	146	146	146
	K	mmol/L	4.43	4.32	4.08
	CL	mEq/L	124	129	107
	CA	mg/dL	11.3	10.7	10.2
	IP	mg/dL	7.1	5.1	5.6
	GLU	mg/dL	111	107	122
	HAPT	mg/dL	33.1	378.0	228.6

(-)-No Units for Test

FOUR WEEK ORAL DOSE RANGE-FINDING STUDY OF WR242511 IN DOGS

INDIVIDUAL ANIMAL CLINICAL CHEMISTRY DATA

STUDY ID: 133
STUDY NO: 133

SEX: FEMALE

GROUP: 3-F : 1.5 mg base/kg/day

ANIMAL ID	TEST	UNITS	DAY-7/-8	DAY 14	DAY 28
7999	ALT	U/L	51	42	35
	AST	U/L	50	46	47
	TP	g/dL	7.0	7.4	6.7
	ALB	g/dL	3.7	3.1	3.0
	GLOB	g/dL	3.3	4.3	3.7
	A/G	-	1.12	0.72	0.81
	TBILI	mg/dL	0.31	0.19	0.17
	ALKP	U/L	96	201	145
	GGT	U/L	4	4	5
	CHOL	mg/dL	217	336	210
	TRY	mg/dL	58	93	59
	LDH	U/L	136	173	54
	CK	U/L	337	139	153
	BUN	mg/dL	23.2	12.0	18.6
	CREA	mg/dL	0.87	0.87	0.79
	NA	mmol/L	144	146	145
	K	mmol/L	4.26	4.59	4.59
	CL	mEq/L	127	129	120
	CA	mg/dL	10.5	10.2	10.0
	IP	mg/dL	4.6	5.7	4.9
	GLU	mg/dL	107	117	114
	HAPT	mg/dL	--X	566.4	244.4

(--)-Data Unavailable
(-)-No Units for Test

X -Below limit of Detection.

FOUR WEEK ORAL DOSE RANGE-FINDING STUDY OF WR242511 IN DOGS

INDIVIDUAL ANIMAL HEMATOLOGY DATA

STUDY ID: 133
STUDY NO: 133
SEX: MALE
GROUP: 1-M : 0.5 mg base/kg/day

ANIMAL ID	TEST	UNITS	Day-7/-8	Day 14	Day 28
7952	RBC	10^6/cmm	6.90	7.49	5.62
	HGB	g/dL	17.2	18.9	14.0
	HCT	%	48.2	53.5	41.3
	MCV	fL	69.9	71.4	73.5
	MCH	pg	24.9	25.2	24.9
	MCHC	g/dl	35.7	35.3	33.9
	RETICS	%RBCs	0.4	1.6	1.1
	NRBC	COUNT	0	1	2
	HB	%	0.0	0.3	0.0
	PLT	10^3/ccm	288	99	131
	PT	sec	7.2	7.0	7.1
	APTT	sec	11.5	10.8	11.1
	WBC	10^3/cmm	8.4	9.3	9.2
	M. Neutrop	10^3/cmm	5.1	6.0	7.5
	I. Neutrop	10^3/cmm	0.0	0.3	0.0
	Lymphocyte	10^3/cmm	2.7	1.9	1.1
	Monocytes	10^3/cmm	0.5	1.0	0.6
	Eosinophil	10^3/cmm	0.1	0.1	0.1
	Basophils	10^3/cmm	0.0	0.0	0.0
	Atypical L	10^3/cmm	0.0	0.0	0.0
MORPHOLOGY OBSERVATIONS:			Anisocytosis Moderate Polychromasia,Slight	Polychromasia,Slight Anisocytosis,Slight	Anisocytosis,Slight Large Platelets Slight

WBC corrected for NRBC = or > 10

FOUR WEEK ORAL DOSE RANGE-FINDING STUDY OF WR242511 IN DOGS

INDIVIDUAL ANIMAL HEMATOLOGY DATA

STUDY ID: 133
STUDY NO: 133
SEX: FEMALE
GROUP: 1-F : 0.5 mg base/kg/day

ANIMAL ID	TEST	UNITS	Day-7/-8	Day 14	Day 28
8017	RBC	10^6/cmm	6.34	6.08	6.19
	HGB	g/dL	15.9	15.2	15.3
	HCT	%	44.0	42.5	43.8
	MCV	fL	69.4	69.9	70.8
	MCH	pg	25.1	25.0	24.7
	MCHC	g/dl	36.1	35.8	34.9
	RETICS	%RBCs	0.7	1.2	0.9
	NRBC	COUNT	0	0	0
	HB	%	0.2	0.2	0.2
	PLT	10^3/ccm	352	246	258
	PT	sec	8.0	7.7	7.9
	APTT	sec	12.3	11.8	11.9
	WBC	10^3/cmm	7.6	7.4	7.4
	M. Neutrop	10^3/cmm	4.3	4.1	3.5
	I. Neutrop	10^3/cmm	0.0	0.1	0.0
	Lymphocyte	10^3/cmm	2.5	2.4	3.1
	Monocytes	10^3/cmm	0.3	0.7	0.6
	Eosinophil	10^3/cmm	0.5	0.1	0.2
	Basophils	10^3/cmm	0.0	0.0	0.0
	Atypical L	10^3/cmm	0.0	0.0	0.0
MORPHOLOGY OBSERVATIONS:			Anisocytosis,Slight Polychromasia,Slight	Polychromasia,Slight Anisocytosis,Slight	Anisocytosis,Slight Polychromasia,Slight Poikilocytes,Slight

WBC corrected for NRBC = or > 10

FOUR WEEK ORAL DOSE RANGE-FINDING STUDY OF WR242511 IN DOGS

INDIVIDUAL ANIMAL HEMATOLOGY DATA

STUDY ID: 133
STUDY NO: 133
SEX: MALE
GROUP: 2-M : 0.9 mg base/kg/day

ANIMAL ID	TEST	UNITS	Day-7/-8	Day 14	Day 28
7951	RBC	10^6/cmm	7.47	5.95	6.71
	HGB	g/dL	18.4	15.2	16.6
	HCT	%	52.2	42.8	48.9
	MCV	fL	69.9	71.9	72.9
	MCH	pg	24.6	25.5	24.7
	MCHC	g/dl	35.2	35.5	33.9
	RETICS	%RBCs	1.3	0.9	2.5
	NRBC	COUNT	2	5	0
	HB	%	0.2	0.2	0.0
	PLT	10^3/ccm	379	103	146
	PT	sec	7.1	6.9	7.1
	APTT	sec	11.5	11.8	11.4
	WBC	10^3/cmm	9.0	7.2	6.6
	M. Neutrop	10^3/cmm	5.1	3.0	3.6
	I. Neutrop	10^3/cmm	0.0	0.1	0.1
	Lymphocyte	10^3/cmm	3.1	3.1	2.3
	Monocytes	10^3/cmm	0.5	0.6	0.5
	Eosinophil	10^3/cmm	0.3	0.3	0.1
	Basophils	10^3/cmm	0.0	0.0	0.0
	Atypical L	10^3/cmm	0.0	0.0	0.0
MORPHOLOGY OBSERVATIONS:			Anisocytosis,Slight	Polychromasia,Slight Anisocytosis,Slight	Anisocytosis,Slight Decreased Platelets Slight

WBC corrected for NRBC = or > 10

FOUR WEEK ORAL DOSE RANGE-FINDING STUDY OF WR242511 IN DOGS

INDIVIDUAL ANIMAL HEMATOLOGY DATA

STUDY ID: 133
STUDY NO: 133
SEX: FEMALE
GROUP: 2-F : 0.9 mg base/kg/day

ANIMAL ID	TEST	UNITS	Day-7/-8	Day 14	Day 28
8000	RBC	10^6/cmm	6.66	5.99	6.28
	HGB	g/dL	16.5	15.1	15.5
	HCT	%	45.8	41.6	44.7
	MCV	fL	68.8	69.4	71.2
	MCH	pg	24.8	25.2	24.7
	MCHC	g/dl	36.0	36.3	34.7
	RETICS	%RBCs	0.6	1.2	2.2
	NRBC	COUNT	0	4	6
	HB	%	0.0	0.0	0.0
	PLT	10^3/ccm	417	66	145
	PT	sec	7.4	7.2	7.5
	APTT	sec	13.0	12.6	13.1
	WBC	10^3/cmm	9.3	10.2	10.3
	M. Neutrop	10^3/cmm	5.8	5.8	7.4
	I. Neutrop	10^3/cmm	0.0	0.2	0.0
	Lymphocyte	10^3/cmm	3.2	2.7	2.4
	Monocytes	10^3/cmm	0.3	1.2	0.5
	Eosinophil	10^3/cmm	0.1	0.3	0.0
	Basophils	10^3/cmm	0.0	0.0	0.0
	Atypical L	10^3/cmm	0.0	0.0	0.0
MORPHOLOGY OBSERVATIONS:			Anisocytosis Moderate Polychromasia,Slight	Polychromasia,Slight Anisocytosis,Slight	Anisocytosis,Slight Polychromasia,Slight

WBC corrected for NRBC = or > 10

FOUR WEEK ORAL DOSE RANGE-FINDING STUDY OF WR242511 IN DOGS

INDIVIDUAL ANIMAL HEMATOLOGY DATA

STUDY ID: 133
STUDY NO: 133
SEX: MALE
GROUP: 3-M : 1.5 mg base/kg/day

ANIMAL ID	TEST	UNITS	Day-7/-8	Day 14	Day 28
7950	RBC	10^6/cmm	6.19	5.11	5.84
	HGB	g/dL	15.6	13.2	14.8
	HCT	%	43.7	37.5	43.7
	MCV	fL	70.6	73.4	74.8
	MCH	pg	25.2	25.8	25.3
	MCHC	g/dl	35.7	35.2	33.9
	RETICS	%RBCs	0.7	1.8	1.3
	NRBC	COUNT	0	1	2
	HB	%	0.0	0.3	0.1
	PLT	10^3/ccm	380	75	143
	PT	sec	7.6	7.1	7.5
	APTT	sec	12.1	12.6	9.9
	WBC	10^3/cmm	10.5	15.5	20.1
	M. Neutrop	10^3/cmm	5.6	11.3	12.9
	I. Neutrop	10^3/cmm	0.0	0.0	0.2
	Lymphocyte	10^3/cmm	3.9	2.6	4.6
	Monocytes	10^3/cmm	0.4	1.4	1.4
	Eosinophil	10^3/cmm	0.6	0.2	0.2
	Basophils	10^3/cmm	0.0	0.0	0.0
	Atypical L	10^3/cmm	0.0	0.0	0.8
MORPHOLOGY OBSERVATIONS:			Anisocytosis Moderate Polychromasia,Slight	Polychromasia,Slight Anisocytosis,Slight	Anisocytosis,Slight

WBC corrected for NRBC = or > 10

FOUR WEEK ORAL DOSE RANGE-FINDING STUDY OF WR242511 IN DOGS

INDIVIDUAL ANIMAL HEMATOLOGY DATA

STUDY ID: 133
STUDY NO: 133
GROUP: 3-F : 1.5 mg base/kg/day
SEX: FEMALE

ANIMAL ID	TEST	UNITS	Day-7/-8	Day 14	Day 28
7999	RBC	10^6/cmm	7.43	5.79	6.35
	HGB	g/dL	18.3	14.7	15.1
	HCT	%	50.2	40.6	45.0
	MCV	fL	67.6	70.1	70.9
	MCH	pg	24.6	25.4	23.8
	MCHC	g/dl	36.5	36.2	33.6
	RETICS	%RBCs	0.5	1.9	1.7
	NRBC	COUNT	0	3	9
	HB	%	0.0	0.0	0.0
	PLT	10^3/ccm	295	28	169
	PT	sec	7.3	6.7	7.1
	APTT	sec	10.9	11.2	11.1
	WBC	10^3/cmm	7.9	9.0	12.7
	M. Neutrop	10^3/cmm	5.2	6.3	8.3
	I. Neutrop	10^3/cmm	0.0	0.0	0.1
	Lymphocyte	10^3/cmm	2.2	1.4	2.9
	Monocytes	10^3/cmm	0.2	1.3	0.8
	Eosinophil	10^3/cmm	0.2	0.0	0.6
	Basophils	10^3/cmm	0.0	0.0	0.0
	Atypical L	10^3/cmm	0.0	0.0	0.0
	MORPHOLOGY OBSERVATIONS:		Anisocytosis,Slight	Anisocytosis,Slight	Anisocytosis,Slight Polychromasia,Slight Poikilocytes,Slight Target Cells,Slight

WBC corrected for NRBC = or > 10

FOUR WEEK ORAL DOSE RANGE-FINDING
STUDY OF WR242511 IN DOGS

INDIVIDUAL ANIMAL METHEMOGLOBIN DATA
TEST: Methemoglobin

STUDY ID: 133
STUDY NO: 133
ABBR: METH

SEX: MALE

UNITS: %

ANIMAL ID	Day-7/-8	Day 0	Day 7	Day 14	Day21-0h	Day21-2h	Day21-8h	Day 28
GROUP: 1-M:0.5 mg base/kg/day								
7952	1.0	0.9	16.1	20.2	16.4	15.7	16.8	12.8
MEAN	1.0	0.9	16.1	20.2	16.4	15.7	16.8	12.8
SD	NA	NA	NA	NA	NA	NA	NA	NA
N	1	1	1	1	1	1	1	1
GROUP: 2-M:0.9 mg base/kg/day								
7951	1.0	0.7	23.9	28.0	24.6	23.8	24.8	20.8
MEAN	1.0	0.7	23.9	28.0	24.6	23.8	24.8	20.8
SD	NA	NA	NA	NA	NA	NA	NA	NA
N	1	1	1	1	1	1	1	1
GROUP: 3-M:1.5 mg base/kg/day								
7950	0.8	0.9	37.1	36.8	33.6	32.4	33.2	29.4
MEAN	0.8	0.9	37.1	36.8	33.6	32.4	33.2	29.4
SD	NA	NA	NA	NA	NA	NA	NA	NA
N	1	1	1	1	1	1	1	1

NA-Not Applicable

FOUR WEEK ORAL DOSE RANGE-FINDING
STUDY OF WR242511 IN DOGS

INDIVIDUAL ANIMAL METHEMOGLOBIN DATA
TEST: Methemoglobin

STUDY ID: 133
STUDY NO: 133
ABBR: METH

SEX: FEMALE

UNITS: %

ANIMAL ID	Day-7/-8	Day 0	Day 7	Day 14	Day21-0h	Day21-2h	Day21-8h	Day 28
GROUP: 1-F:0.5 mg base/kg/day								
8017	0.7	0.6	12.8	15.4	13.2	13.1	13.7	11.4
MEAN	0.7	0.6	12.8	15.4	13.2	13.1	13.7	11.4
SD	NA	NA	NA	NA	NA	NA	NA	NA
N	1	1	1	1	1	1	1	1
GROUP: 2-F:0.9 mg base/kg/day								
8000	0.5	0.7	22.8	30.3	28.1	25.9	26.1	23.9
MEAN	0.5	0.7	22.8	30.3	28.1	25.9	26.1	23.9
SD	NA	NA	NA	NA	NA	NA	NA	NA
N	1	1	1	1	1	1	1	1
GROUP: 3-F:1.5 mg base/kg/day								
7999	0.8	0.6	28.0	37.9	32.8	32.4	32.9	29.4
MEAN	0.8	0.6	28.0	37.9	32.8	32.4	32.9	29.4
SD	NA	NA	NA	NA	NA	NA	NA	NA
N	1	1	1	1	1	1	1	1

NA-Not Applicable

Table 9

FOUR WEEK ORAL DOSE RANGE-FINDING STUDY OF WR242511 IN DOGS

Summary of Microscopic Lesions[a]

MICROSCOPIC LESIONS		Dose (mg base/kg/day)		
ORGAN - lesion	Sex	0.5	0.9	1.5
LUNGS				
- Proteinic alveolar exudate	M	1	0	1
	F	0	1	1
- Acute alveolar inflammation	M	1	0	2
	F	0	1	2
- Macrophage infiltrates	M	1	0	2
	F	0	2	2
LIVER				
- Hepatocellular swelling	M	0	1	1
	F	1	1	1

[a]Lesion severity was scored as follows:

1 = Minimal 3 = Moderate
2 = Mild 4 = Marked

For additional information see Pathology Report in Appendix 3.

APPENDIX 1

Clinical Pathology Methodology

Clinical Chemistry Test Directory

STUDY: 133

NO.	ABBR. UNITS	DESCRIPTION PRECISION	CALCULATED	OPERAND A	OPERAND B	---LOWER LIMIT--- MALE	FEMALE	---UPPER LIMIT--- MALE	FEMALE
1.	ALT U/L	Alanine Aminotransferase Integer	NO			20	20	60	60
2.	AST U/L	Aspartate Aminotransferase Integer	NO			20	20	60	60
3.	TP g/dL	Total Protein 0.0	NO			6.0	6.0	8.0	8.0
4.	ALB g/dL	Albumin 0.0	NO			2.7	2.7	3.7	3.7
5.	TBILI mg/dL	Total Bilirubin 0.00	NO			0.00	0.00	0.30	0.30
6.	ALKP U/L	Alkaline Phosphatase Integer	NO			50	50	200	200
7.	GGT U/L	Gamma Glutamyl Transferase Integer	NO			0	0	10	10
8.	CHOL mg/dL	Cholesterol Integer	NO			150	150	250	250
9.	TRY mg/dL	Triglycerides Integer	NO			20	20	60	60
10.	LDH U/L	Lactate Dehydrogenase Integer	NO			25	25	200	200
11.	CK U/L	Creatine Kinase Integer	NO			50	50	300	300
12.	BUN mg/dL	Blood Urea Nitrogen 0.0	NO			8.0	8.0	18.0	18.0
13.	CREA mg/dL	Creatinine 0.00	NO			0.50	0.50	1.00	1.00
14.	NA mmol/L	Sodium Integer	NO			140	140	150	150
15.	K mmol/L	Potassium 0.00	NO			4.00	4.00	5.25	5.25

(REPORT CONTINUED)

Clinical Chemistry Test Directory

STUDY: 133

NO.	ABBR. / UNITS	DESCRIPTION / PRECISION	CALCULATED	OPERAND A	OPERAND B	---LOWER LIMIT--- MALE	FEMALE	---UPPER LIMIT--- MALE	FEMALE
16.	CL mEq/L	Chloride Integer	NO			110.0	110.0	130.0	130.0
17.	CA mg/dL	Calcium 0.0	NO			9.0	9.0	12.0	12.0
18.	IP mg/dL	Inorganic Phosphorus 0.0	NO			4.0	4.0	8.0	8.0
19.	GLU mg/dL	Glucose Integer	NO			90	90	140	140
20.	HAPT mg/dL	Haptoglobin 0.0	NO			0	0	250	250
21.	GLOB g/dL	Globulin 0.0	Operand A - Operand B	TP	ALB	3.0	3.0	6.0	6.0
22.	A/G -	A/G Ratio 0.00	Operand A / Operand B	ALB	GLOB	0.50	0.50	1.50	1.50

(END OF REPORT) 02-JUN-1994

CLINICAL CHEMISTRY

Alanine Aminotransferase (ALT/GPT)

Modified Wroblewski & La Due procedure
Ciba-Corning 550 Express Clinical Chemistry System
Henry, R.J., Chiamori, N., Golub, O.J. and Berkman, S.
Am. J. Clin. Path., 34, 381, 1960.

Aspartate Aminotransferase (AST/GOT)

Modified Karmen procedure
Ciba-Corning 550 Express Clinical Chemistry System
Bergmeyer, H.V., Scheibe, P., and Wahlefeld, A.W.
Clin. Chem., 24, 58, 1978.

Total Protein

Biuret technique
Ciba-Corning 550 Express Clinical Chemistry System
Kingsley, G.R.
J. Biol. Chem. 131, 197, 1939.

Albumin

Bromocresol green method
Ciba-Corning 550 Express Clinical Chemistry System
Doumas, B.T. and Biggs, H.G.
Standard Methods of Clinical Chemistry, 7, 175, 1972.

Total Bilirubin

Modified Walters and Gerard method
Ciba-Corning 550 Express Clinical Chemistry System
Ertinghausen G., Fabiny-Byrd, D.L., Tiffany, T.O., and Carey, S.J.
Clinical Chem., 19, 1366, 1973.

Alkaline Phosphatase

Modified Bessey-Lowry procedure
Ciba-Corning 550 Express Clinical Chemistry System
Neumann, H. and Von Vreedendaal
M. Clin. Chem. Acta., 17, 183, 1967.

Gamma Glutamyl Transferase (GGT)

JFCC Methods for Gamms Glutamyl Transferase
Shaw, L.M., Stromme, J.H., London, J.L., Theodorsen, L.
J. Clin. Chem. C;in, Biochem. 21 (1983) 633-646

Cholesterol

Cholesterol esterase-oxidase method
Ciba-Corning 550 Express Clinical Chemistry System
Rosechlow, P., et. al
Z.F. Klin. Chem. V. Klin. Biochem. 12, 226, 1974.

CLINICAL CHEMISTRY (Contd.)

Triglycerides

Tetrazolium salt reduction method
Ciba-Corning 550 Express Clinical Chemistry System
Klotzsch, S., et. al.
Advances Automated Analysis, Vol. 1, Mediad Inc., Tarrytown, N.Y., p. 111, 1973.

Lactate Dehydrogenase

L → P technique
Ciba-Corning 550 Express Clinical Chemistry System
Wacker, W.E.C., Ulmer, D.D., Valle, B.L.,
New England J Med. 225, 449, 1956

Creatine Kinase (CK)

Modification of Szasz *et al.* procedure
Ciba-Corning 550 Express Clinical Chemistry System
Clin. Chem. 22 650-656, 1976.

Urea Nitrogen (BUN)

Modified urease technique
Ciba-Corning 550 Express Clinical Chemistry System
Talke, H. and Schubert, G.E.
Klin. Wchnschr. 43, 174, 1965.

Creatinine

Jaffe method
Ciba-Corning 550 Express Clinical Chemistry System
Larsen. K.
Clin. Chem. Acta, 41, 209, 1972

Na+, K+

Ion specific electrodes
Model 614 ISE Na+/K+ Analyzer (Ciba Corning)

Chloride

Mecuric thiocyanate procedure
Ciba-Corning 550 Express Clinical Chemistry System
Zall, O.M., Fisher, D. and Garner, M.Q.
Anal. Chem, 28, 1065, 1956.

Calcium

Modified alizarin procedure
Ciba-Corning 550 Express Clinical Chemistry System
Frings, C.S., et. al.
Clin. Chem., 16, 816, 1970.

Phosphorus, Inorganic

Ammonium molybdate method
Ciba-Corning 550 Express Clinical Chemistry System
Fiske, C.H. and Subbarow, Y.
J. Biol. Chem. 66, 325, 1925.

CLINICAL CHEMISTRY (Contd.)

Glucose

Hexokinase method
Ciba-Corning 550 Express Clinical Chemistry System
Bondar, J.L. and Mead, D.C.
Clin. Chem. 20, 586, 1974.

Haptoglobin

Antigen-antibody method
Ciba-Corning 550 Express Clinical Chemistry System
Atlantic Antibodies Test Kit

Hematology Test Directory

STUDY: 133

NO.	ABBR. UNITS	DESCRIPTION PRECISION	CALCULATED	OPERAND A	OPERAND B	---LOWER LIMIT--- MALE	FEMALE	---UPPER LIMIT--- MALE	FEMALE
1.	RBC 10^6/cmm	Erythrocytes 0.00	NO			6.00	6.00	8.00	8.00
2.	HGB g/dL	Hemoglobin 0.0	NO			12.0	12.0	19.0	19.0
3.	HCT %	Hematocrit 0.0	NO			35.0	35.0	55.0	55.0
4.	MCV fL	Mean Corpuscular Volume 0.0	NO			57.0	57.0	70.0	70.0
5.	MCH pg	Mean Corpuscular Hemo. 0.0	NO			20.	20	25	25
6.	MCHC g/dl	Mean Corpus. Hemo. Conc. 0.0	NO			32.0	32.0	38.0	38.0
7.	RETICS %RBCs	Reticulocytes 0.0	NO			0.0	0.0	1.0	1.0
8.	HB %	Heinz Bodies 0.0	NO			0.0	0.0	2.0	2.0
9.	HJ %	Howell-Jolly Bodies 0.0	NO			0.0	0.0	2.0	2.0
10.	PLT 10^3/ccm	Platelets Integer	NO			200	200	500	500
11.	PT sec	Prothrombin Time 0.0	NO			6.0	6.0	9.0	9.0
12.	APTT sec	Act. Partial Thrombo. Time 0.0	NO			7.0	7.0	12.0	12.0
13.	FIBR mg/dL	Fibrinogen Integer	NO						
14.	WBC 10^3/cmm	Leukocytes 0.0	NO			7.0	7.0	15.0	15.0
15.	METH %	Methemoglobin 0.0	NO			0	0	3	3

(END OF REPORT) 29-JUN-1994

HEMATOLOGY

Erythrocyte Count
Electronic counting procedure
Sysmex 180A Hematology Analyzer

Hemoglobin
Cyanomethemoglobin method
Sysmex 180A Hematology Analyzer

Hematocrit
Indirect method; calculated value based on volume of red cells and volume of blood

Mean Corpuscular Volume (MCV)
Indirect method; calculated value based on hematocrit and red blood cell count

Mean Corpuscular Hemoglobin (MCH)
Indirect method; calculated value based on erythrocyte count and hemoglobin

Mean Corpuscular Hemoglobin Concentration (MCHC)
Indirect method; calculated value based on hematocrit and hemoglobin

Reticulocyte Count
New methylene blue staining procedure
Brecher, G., Am. J. Clin. Path., 19, 895, 1949.

Heinz Bodies
Methyl Violet staining technique

Platelet Count
Electronic counting procedure
Sysmex 180A Hematology Analyzer

Prothrombin Time (PT)
Electra 700 coagulation machine

Activated Partial Thromboplastin Time (APTT)
Electra 700 coagulation machine

Fibrinogen
Electra 700 coagulation machine

Leukocyte Count
Electronic counting procedure
Sysmex 180A Hematology Analyzer

Methemoglobin
Measured with a Co-oximeter (Instrumentation Laboratory Model 282)

Leukocyte Differential Count

Neutrophils - Immature (bands)
Neutrophils - Mature (segs)
Monocytes
Basophils
Lymphocytes
Eosinophils
Wright stain procedure

Schalm, O.W., Jain, N.C. and Carroll, E.J. Veterinary Hematology, Color Plates Chapter, 3rd Edition, Lee and Febiger, 1975.

APPENDIX 2

Individual Observations (Clinical Signs)

FOUR WEEK ORAL DOSE RANGE-FINDING
STUDY OF WR24511 IN DOGS

INDIVIDUAL CLINICAL SIGNS

STUDY: 133 GROUP: 1-M SEX: MALE
DAY 0-DAY 28 DOSE: 0.5(mg/kg)

ANIMAL #	OBSERVATIONS	SEVERITY	LOC	ONSET	DURATION	FREQUENCY
7952	Blue Gums	1		DAY 7	DAY 25	7
	Blue Sclera	1		DAY 9	DAY 27	16
	Blue Tongue	1		DAY 8	DAY 26	10
	Blue Tongue	2		DAY 7	DAY 11	2
	Normal			DAY 0	DAY 15	9
	Scheduled Sacrifice			DAY 28	DAY 28	1
	Vomit Seen In Run			DAY 3	DAY 25	7

GROUP: 2-M SEX: MALE
DOSE: 0.9(mg/kg)

ANIMAL #	OBSERVATIONS	SEVERITY	LOC	ONSET	DURATION	FREQUENCY
7951	Blue Gums	1		DAY 8	DAY 26	6
	Blue Sclera	1		DAY 8	DAY 27	20
	Blue Tongue	1		DAY 6	DAY 27	12
	Blue Tongue	2		DAY 8	DAY 24	3
	Normal			DAY 0	DAY 5	6
	Pale Gums			DAY 12	DAY 25	7
	Pale Tongue			DAY 9	DAY 25	7
	Scheduled Sacrifice			DAY 28	DAY 28	1

GROUP: 3-M SEX: MALE
DOSE: 1.5(mg/kg)

ANIMAL #	OBSERVATIONS	SEVERITY	LOC	ONSET	DURATION	FREQUENCY
7950	Blue Gums	1		DAY 6	DAY 7	2
	Blue Sclera	1		DAY 11	DAY 27	14
	Blue Tongue	1		DAY 2	DAY 22	8
	Blue Tongue	2		DAY 7	DAY 20	2
	Normal			DAY 0	DAY 1	2
	Pale Gums			DAY 3	DAY 27	22
	Pale Tongue			DAY 8	DAY 27	16
	Scheduled Sacrifice			DAY 28	DAY 28	1

Severity Codes

Observations	Severity No.	Description
Blue Gums/	1	Slight (barely perceptible, slight blue tinged color)
Blue Tongue/	2	Moderate (easily seen, blue color)
Blue Sclera	3	Severe (marked, deep blue-purple color)

FOUR WEEK ORAL DOSE RANGE-FINDING
STUDY OF WR24511 IN DOGS

INDIVIDUAL CLINICAL SIGNS

STUDY: 133 GROUP: 1-F SEX: FEMALE
DAY 0-DAY 28 DOSE: 0.5(mg/kg)

ANIMAL #	OBSERVATIONS	SEVERITY	LOC	ONSET	DURATION	FREQUENCY
8017	Blue Gums	1		DAY 24	DAY 26	2
	Blue Sclera	1		DAY 6	DAY 27	22
	Blue Tongue	1		DAY 8	DAY 27	10
	Diarrhea	2		DAY 10	DAY 10	1
	Normal			DAY 0	DAY 5	6
	Pale Gums			DAY 15	DAY 23	4
	Pale Tongue			DAY 13	DAY 23	5
	Scheduled Sacrifice			DAY 28	DAY 28	1

GROUP: 2-F SEX: FEMALE
DOSE: 0.9(mg/kg)

ANIMAL #	OBSERVATIONS	SEVERITY	LOC	ONSET	DURATION	FREQUENCY
8000	Blue Gums	1		DAY 6	DAY 22	14
	Blue Gums	2		DAY 27	DAY 27	1
	Blue Sclera	1		DAY 6	DAY 26	20
	Blue Sclera	2		DAY 27	DAY 27	1
	Blue Tongue	1		DAY 3	DAY 23	6
	Blue Tongue	2		DAY 6	DAY 27	16
	Blue Tongue	3		DAY 12	DAY 20	2
	Normal			DAY 0	DAY 5	4
	Pale Gums			DAY 15	DAY 26	6
	Scheduled Sacrifice			DAY 28	DAY 28	1

GROUP: 3-F SEX: FEMALE
DOSE: 1.5(mg/kg)

ANIMAL #	OBSERVATIONS	SEVERITY	LOC	ONSET	DURATION	FREQUENCY
7999	Blue Gums	1		DAY 5	DAY 27	15
	Blue Gums	2		DAY 25	DAY 25	1
	Blue Sclera	1		DAY 5	DAY 27	22
	Blue Tongue	1		DAY 1	DAY 19	13
	Blue Tongue	2		DAY 6	DAY 27	11
	Blue Tongue	3		DAY 8	DAY 21	3
	Normal			DAY 0	DAY 0	1
	Pale Gums			DAY 15	DAY 19	4
	Scheduled Sacrifice			DAY 28	DAY 28	1

Severity Codes

Observations	Severity No.	Description
Blue Gums/	1	Slight (barely perceptible, slight blue tinged color)
Blue Tongue/	2	Moderate (easily seen, blue color)
Blue Sclera	3	Severe (marked, deep blue-purple color)

SUMMARY OF OBSERVATION INCIDENCE

STUDY: 133 SEX: MALE

PERIOD	DOSE:(mg/kg) GROUP:	0.5 1-M	0.9 2-M	1.5 3-M
DAY 0				
No. Observed		1	1	1
Normal		1 100%	1 100%	1 100%
DAY 1				
No. Observed		1	1	1
Normal		1 100%	1 100%	1 100%
DAY 2				
No. Observed		1	1	1
Normal		1 100%	1 100%	0
Blue Tongue				
SEV				
1		0	0	1 100%
DAY 3				
No. Observed		1	1	1
Normal		0	1 100%	0
Blue Tongue				
SEV				
1		0	0	1 100%
Vomit Seen In Run		1 100%	0	0
Pale Gums		0	0	1 100%
DAY 4				
No. Observed		1	1	1
Normal		1 100%	1 100%	0
Blue Tongue				
SEV				
1		0	0	1 100%
Pale Gums		0	0	1 100%
DAY 5				
No. Observed		1	1	1
Normal		1 100%	1 100%	0
Blue Tongue				
SEV				
1		0	0	1 100%

Severity Codes

Observations	Severity No.	Description
Blue Gums/	1	Slight (barely preceptible, slight blue tinged color
Blue Tongue/	2	Moderate (easily seen, blue color)
Blue Sclera	3	Severe (marked, deep blue-purple color)

SUMMARY OF OBSERVATION INCIDENCE

STUDY: 133 SEX: MALE

PERIOD	DOSE:(mg/kg) GROUP:	0.5 1-M	0.9 2-M	1.5 3-M
DAY 6				
No. Observed		1	1	1
Normal		1 100%	0	0
Blue Gums				
SEV				
1		0	0	1 100%
Blue Tongue				
SEV				
1		0	1 100%	1 100%
DAY 7				
No. Observed		1	1	1
Blue Gums				
SEV				
1		1 100%	0	1 100%
Blue Tongue				
SEV				
1		0	1 100%	0
2		1 100%	0	1 100%
DAY 8				
No. Observed		1	1	1
Blue Gums				
SEV				
1		0	1 100%	0
Blue Sclera				
SEV				
1		0	1 100%	0
Blue Tongue				
SEV				
1		1 100%	0	0
2		0	1 100%	0
Pale Gums		0	0	1 100%
Pale Tongue		0	0	1 100%

Severity Codes

Observations	Severity No.	Description
Blue Gums/	1	Slight (barely preceptible, slight blue tinged color
Blue Tongue/	2	Moderate (easily seen, blue color)
Blue Sclera	3	Severe (marked, deep blue-purple color)

FOUR WEEK ORAL DOSE RANGE-FINDING STUDY OF WR24511 IN DOGS

SUMMARY OF OBSERVATION INCIDENCE

STUDY: 133 SEX: MALE

PERIOD	DOSE:(mg/kg) GROUP:	0.5 1-M	0.9 2-M	1.5 3-M
DAY 9				
	No. Observed	1	1	1
	Blue Gums			
	SEV			
	1	1 100%	0	0
	Blue Sclera			
	SEV			
	1	1 100%	1 100%	0
	Pale Gums	0	0	1 100%
	Pale Tongue	0	1 100%	1 100%
DAY 10				
	No. Observed	1	1	1
	Blue Gums			
	SEV			
	1	1 100%	0	0
	Blue Sclera			
	SEV			
	1	1 100%	1 100%	0
	Blue Tongue			
	SEV			
	1	1 100%	1 100%	0
	Vomit Seen In Run	1 100%	0	0
	Pale Gums	0	0	1 100%
	Pale Tongue	0	0	1 100%
DAY 11				
	No. Observed	1	1	1
	Blue Gums			
	SEV			
	1	1 100%	0	0
	Blue Sclera			
	SEV			
	1	1 100%	1 100%	1 100%
	Blue Tongue			
	SEV			
	2	1 100%	0	0
	Vomit Seen In Run	1 100%	0	0
	Pale Gums	0	0	1 100%
	Pale Tongue	0	1 100%	1 100%

Severity Codes

Observations	Severity No.	Description
Blue Gums/	1	Slight (barely preceptible, slight blue tinged color
Blue Tongue/	2	Moderate (easily seen, blue color)
Blue Sclera	3	Severe (marked, deep blue-purple color)

SUMMARY OF OBSERVATION INCIDENCE

STUDY: 133 SEX: MALE

PERIOD	DOSE:(mg/kg) GROUP:	0.5 1-M	0.9 2-M	1.5 3-M
DAY 12				
No. Observed		1	1	1
Normal		1 100%	0	0
Blue Sclera				
SEV				
1		0	1 100%	1 100%
Pale Gums		0	1 100%	1 100%
Pale Tongue		0	1 100%	1 100%
DAY 13				
No. Observed		1	1	1
Blue Gums				
SEV				
1		0	1 100%	0
Blue Sclera				
SEV				
1		1 100%	1 100%	1 100%
Blue Tongue				
SEV				
1		0	1 100%	0
Pale Gums		0	0	1 100%
Pale Tongue		0	0	1 100%
DAY 14				
No. Observed		1	1	1
Normal		1 100%	0	0
Blue Gums				
SEV				
1		0	1 100%	0
Blue Sclera				
SEV				
1		0	1 100%	1 100%
Blue Tongue				
SEV				
1		0	1 100%	0
Pale Gums		0	0	1 100%
Pale Tongue		0	0	1 100%

Severity Codes

Observations	Severity No.	Description
Blue Gums/	1	Slight (barely preceptible, slight blue tinged color
Blue Tongue/	2	Moderate (easily seen, blue color)
Blue Sclera	3	Severe (marked, deep blue-purple color)

SUMMARY OF OBSERVATION INCIDENCE

STUDY: 133 SEX: MALE

PERIOD	DOSE:(mg/kg) GROUP:	0.5 1-M	0.9 2-M	1.5 3-M
DAY 15				
No. Observed		1	1	1
Normal		1 100%	0	0
Blue Sclera				
SEV				
1		0	1 100%	0
Pale Gums		0	1 100%	1 100%
Pale Tongue		0	1 100%	1 100%
DAY 16				
No. Observed		1	1	1
Blue Gums				
SEV				
1		1 100%	0	0
Blue Sclera				
SEV				
1		1 100%	1 100%	0
Blue Tongue				
SEV				
1		1 100%	1 100%	1 100%
Pale Gums		0	0	1 100%
DAY 17				
No. Observed		1	1	1
Blue Sclera				
SEV				
1		1 100%	1 100%	1 100%
Blue Tongue				
SEV				
1		0	1 100%	0
Pale Gums		0	0	1 100%
Pale Tongue		0	0	1 100%

Severity Codes

Observations	Severity No.	Description
Blue Gums/	1	Slight (barely preceptible, slight blue tinged color
Blue Tongue/	2	Moderate (easily seen, blue color)
Blue Sclera	3	Severe (marked, deep blue-purple color)

SUMMARY OF OBSERVATION INCIDENCE

STUDY: 133 SEX: MALE

PERIOD	DOSE:(mg/kg) GROUP:	0.5 1-M	0.9 2-M	1.5 3-M
DAY 18				
No. Observed		1	1	1
Blue Sclera				
SEV				
1		1 100%	1 100%	1 100%
Blue Tongue				
SEV				
1		1 100%	0	0
Vomit Seen In Run		1 100%	0	0
Pale Gums		0	1 100%	1 100%
Pale Tongue		0	1 100%	1 100%
DAY 19				
No. Observed		1	1	1
Blue Sclera				
SEV				
1		1 100%	1 100%	1 100%
Pale Gums		0	1 100%	1 100%
Pale Tongue		0	1 100%	1 100%
DAY 20				
No. Observed		1	1	1
Blue Sclera				
SEV				
1		1 100%	1 100%	1 100%
Blue Tongue				
SEV				
2		0	1 100%	1 100%
Pale Gums		0	0	1 100%
DAY 21				
No. Observed		1	1	1
Blue Sclera				
SEV				
1		1 100%	1 100%	1 100%
Blue Tongue				
SEV				
1		1 100%	1 100%	1 100%
Vomit Seen In Run		1 100%	0	0
Pale Gums		0	1 100%	1 100%

Severity Codes

Observations	Severity No.	Description
Blue Gums/	1	Slight (barely preceptible, slight blue tinged color
Blue Tongue/	2	Moderate (easily seen, blue color)
Blue Sclera	3	Severe (marked, deep blue-purple color)

SUMMARY OF OBSERVATION INCIDENCE

STUDY: 133 SEX: MALE

PERIOD	DOSE:(mg/kg) GROUP:	0.5 1-M	0.9 2-M	1.5 3-M
DAY 22				
	No. Observed	1	1	1
	Blue Sclera			
	SEV			
	1	1 100%	1 100%	1 100%
	Blue Tongue			
	SEV			
	1	1 100%	1 100%	1 100%
	Vomit Seen In Run	1 100%	0	0
	Pale Gums	0	1 100%	1 100%
DAY 23				
	No. Observed	1	1	1
	Blue Gums			
	SEV			
	1	0	1 100%	0
	Blue Sclera			
	SEV			
	1	1 100%	1 100%	0
	Blue Tongue			
	SEV			
	1	1 100%	1 100%	0
	Pale Gums	0	0	1 100%
	Pale Tongue	0	0	1 100%
DAY 24				
	No. Observed	1	1	1
	Blue Gums			
	SEV			
	1	1 100%	1 100%	0
	Blue Sclera			
	SEV			
	1	1 100%	1 100%	1 100%
	Blue Tongue			
	SEV			
	1	1 100%	0	0
	2	0	1 100%	0
	Pale Gums	0	0	1 100%
	Pale Tongue	0	0	1 100%

Severity Codes

Observations	Severity No.	Description
Blue Gums/	1	Slight (barely preceptible, slight blue tinged color
Blue Tongue/	2	Moderate (easily seen, blue color)
Blue Sclera	3	Severe (marked, deep blue-purple color)

FOUR WEEK ORAL DOSE RANGE-FINDING STUDY OF WR24511 IN DOGS

SUMMARY OF OBSERVATION INCIDENCE

STUDY: 133 SEX: MALE

PERIOD	DOSE:(mg/kg) GROUP:	0.5 1-M	0.9 2-M	1.5 3-M
DAY 25				
No. Observed		1	1	1
Blue Gums				
SEV				
1		1 100%	0	0
Blue Sclera				
SEV				
1		1 100%	1 100%	1 100%
Blue Tongue				
SEV				
1		1 100%	0	0
Vomit Seen In Run		1 100%	0	0
Pale Gums		0	1 100%	1 100%
Pale Tongue		0	1 100%	1 100%
DAY 26				
No. Observed		1	1	1
Blue Gums				
SEV				
1		0	1 100%	0
Blue Sclera				
SEV				
1		1 100%	1 100%	1 100%
Blue Tongue				
SEV				
1		1 100%	1 100%	0
Pale Gums		0	0	1 100%
Pale Tongue		0	0	1 100%
DAY 27				
No. Observed		1	1	1
Blue Sclera				
SEV				
1		1 100%	1 100%	1 100%
Blue Tongue				
SEV				
1		0	1 100%	0
Pale Gums		0	0	1 100%
Pale Tongue		0	0	1 100%

Severity Codes

Observations	Severity No.	Description
Blue Gums/	1	Slight (barely preceptible, slight blue tinged color
Blue Tongue/	2	Moderate (easily seen, blue color)
Blue Sclera	3	Severe (marked, deep blue-purple color)

SUMMARY OF OBSERVATION INCIDENCE

STUDY: 133 SEX: MALE

PERIOD	DOSE:(mg/kg) GROUP:	0.5 1-M	0.9 2-M	1.5 3-M
DAY 28				
No. Observed		1	1	1
Scheduled Sacrifice		1 100%	1 100%	1 100%

Severity Codes

Observations	Severity No.	Description
Blue Gums/	1	Slight (barely preceptible, slight blue tinged color
Blue Tongue/	2	Moderate (easily seen, blue color)
Blue Sclera	3	Severe (marked, deep blue-purple color)

FOUR WEEK ORAL DOSE RANGE-FINDING
STUDY OF WR24511 IN DOGS

SUMMARY OF OBSERVATION INCIDENCE

STUDY: 133 SEX: FEMALE

PERIOD	DOSE:(mg/kg) GROUP:	0.5 1-F	0.9 2-F	1.5 3-F
DAY 5				
No. Observed		1	1	1
Normal		1 100%	1 100%	0
Blue Gums				
SEV				
1		0	0	1 100%
Blue Sclera				
SEV				
1		0	0	1 100%
Blue Tongue				
SEV				
1		0	0	1 100%
DAY 6				
No. Observed		1	1	1
Blue Gums				
SEV				
1		0	1 100%	1 100%
Blue Sclera				
SEV				
1		1 100%	1 100%	1 100%
Blue Tongue				
SEV				
2		0	1 100%	1 100%
DAY 7				
No. Observed		1	1	1
Blue Gums				
SEV				
1		0	1 100%	1 100%
Blue Sclera				
SEV				
1		1 100%	1 100%	1 100%
Blue Tongue				
SEV				
1		0	1 100%	0
2		0	0	1 100%

Severity Codes

Observations	Severity No.	Description
Blue Gums/	1	Slight (barely preceptible, slight blue tinged color
Blue Tongue/	2	Moderate (easily seen, blue color)
Blue Sclera	3	Severe (marked, deep blue-purple color)

SUMMARY OF OBSERVATION INCIDENCE

STUDY: 133 SEX: FEMALE

PERIOD	DOSE:(mg/kg) GROUP:	0.5 1-F	0.9 2-F	1.5 3-F
DAY 8				
No. Observed		1	1	1
Blue Gums				
SEV				
1		0	1 100%	1 100%
Blue Sclera				
SEV				
1		1 100%	1 100%	1 100%
Blue Tongue				
SEV				
1		1 100%	0	0
2		0	1 100%	0
3		0	0	1 100%
DAY 9				
No. Observed		1	1	1
Blue Gums				
SEV				
1		0	1 100%	1 100%
Blue Sclera				
SEV				
1		1 100%	1 100%	0
Blue Tongue				
SEV				
2		0	1 100%	1 100%
DAY 10				
No. Observed		1	1	1
Blue Gums				
SEV				
1		0	1 100%	1 100%
Blue Sclera				
SEV				
1		1 100%	1 100%	1 100%
Blue Tongue				
SEV				
2		0	1 100%	1 100%
Diarrhea				
SEV				
2		1 100%	0	0

Severity Codes

Observations	Severity No.	Description
Blue Gums/	1	Slight (barely preceptible, slight blue tinged color
Blue Tongue/	2	Moderate (easily seen, blue color)
Blue Sclera	3	Severe (marked, deep blue-purple color)

SUMMARY OF OBSERVATION INCIDENCE

STUDY: 133 SEX: FEMALE

PERIOD	DOSE:(mg/kg) GROUP:	0.5 1-F	0.9 2-F	1.5 3-F
DAY 11				
No. Observed		1	1	1
Blue Gums				
SEV				
1		0	1 100%	1 100%
Blue Sclera				
SEV				
1		1 100%	1 100%	1 100%
Blue Tongue				
SEV				
1		1 100%	0	0
2		0	1 100%	1 100%
DAY 12				
No. Observed		1	1	1
Blue Gums				
SEV				
1		0	1 100%	1 100%
Blue Sclera				
SEV				
1		1 100%	1 100%	1 100%
Blue Tongue				
SEV				
1		0	0	1 100%
3		0	1 100%	0
DAY 13				
No. Observed		1	1	1
Blue Gums				
SEV				
1		0	1 100%	0
Blue Sclera				
SEV				
1		1 100%	1 100%	1 100%
Blue Tongue				
SEV				
1		0	0	1 100%
2		0	1 100%	0
Pale Tongue		1 100%	0	0

Severity Codes

Observations	Severity No.	Description
Blue Gums/	1	Slight (barely preceptible, slight blue tinged color
Blue Tongue/	2	Moderate (easily seen, blue color)
Blue Sclera	3	Severe (marked, deep blue-purple color)

SUMMARY OF OBSERVATION INCIDENCE

STUDY: 133 SEX: FEMALE

PERIOD	DOSE:(mg/kg) GROUP:	0.5 1-F	0.9 2-F	1.5 3-F
DAY 14				
No. Observed		1	1	1
Blue Sclera				
SEV				
1		1 100%	0	1 100%
Blue Tongue				
SEV				
1		0	1 100%	1 100%
Pale Tongue		1 100%	0	0
DAY 15				
No. Observed		1	1	1
Blue Sclera				
SEV				
1		1 100%	1 100%	1 100%
Blue Tongue				
SEV				
1		0	1 100%	1 100%
Pale Gums		1 100%	1 100%	1 100%
Pale Tongue		1 100%	0	0
DAY 16				
No. Observed		1	1	1
Blue Sclera				
SEV				
1		1 100%	1 100%	1 100%
Blue Tongue				
SEV				
1		0	0	1 100%
2		0	1 100%	0
Pale Gums		1 100%	1 100%	0
Pale Tongue		1 100%	0	0

Severity Codes

Observations	Severity No.	Description
Blue Gums/	1	Slight (barely preceptible, slight blue tinged color
Blue Tongue/	2	Moderate (easily seen, blue color)
Blue Sclera	3	Severe (marked, deep blue-purple color)

SUMMARY OF OBSERVATION INCIDENCE

STUDY: 133 SEX: FEMALE

PERIOD	DOSE:(mg/kg) GROUP:	0.5 1-F	0.9 2-F	1.5 3-F
DAY 17				
No. Observed		1	1	1
Blue Gums				
SEV				
1		0	1 100%	0
Blue Sclera				
SEV				
1		1 100%	1 100%	1 100%
Blue Tongue				
SEV				
1		0	0	1 100%
2		0	1 100%	0
Pale Gums		0	0	1 100%
DAY 18				
No. Observed		1	1	1
Blue Gums				
SEV				
1		0	1 100%	0
Blue Sclera				
SEV				
1		1 100%	1 100%	1 100%
Blue Tongue				
SEV				
1		1 100%	0	1 100%
2		0	1 100%	0
Pale Gums		0	0	1 100%

Severity Codes

Observations	Severity No.	Description
Blue Gums/	1	Slight (barely preceptible, slight blue tinged color
Blue Tongue/	2	Moderate (easily seen, blue color)
Blue Sclera	3	Severe (marked, deep blue-purple color)

SUMMARY OF OBSERVATION INCIDENCE

STUDY: 133 SEX: FEMALE

PERIOD	DOSE:(mg/kg) GROUP:	0.5 1-F	0.9 2-F	1.5 3-F
DAY 19				
No. Observed		1	1	1
Blue Gums				
SEV				
1		0	1 100%	0
Blue Sclera				
SEV				
1		1 100%	1 100%	1 100%
Blue Tongue				
SEV				
1		0	0	1 100%
2		0	1 100%	0
Pale Gums		0	0	1 100%
DAY 20				
No. Observed		1	1	1
Blue Gums				
SEV				
1		0	1 100%	1 100%
Blue Sclera				
SEV				
1		1 100%	1 100%	1 100%
Blue Tongue				
SEV				
1		1 100%	0	0
3		0	1 100%	1 100%
DAY 21				
No. Observed		1	1	1
Blue Gums				
SEV				
1		0	1 100%	1 100%
Blue Sclera				
SEV				
1		1 100%	1 100%	1 100%
Blue Tongue				
SEV				
1		1 100%	0	0
2		0	1 100%	0
3		0	0	1 100%

Severity Codes

Observations	Severity No.	Description
Blue Gums/	1	Slight (barely preceptible, slight blue tinged color
Blue Tongue/	2	Moderate (easily seen, blue color)
Blue Sclera	3	Severe (marked, deep blue-purple color)

SUMMARY OF OBSERVATION INCIDENCE

STUDY: 133 SEX: FEMALE

PERIOD	DOSE:(mg/kg) GROUP:	0.5 1-F	0.9 2-F	1.5 3-F
DAY 22				
No. Observed		1	1	1
Blue Gums				
SEV				
1		0	1 100%	1 100%
Blue Sclera				
SEV				
1		1 100%	1 100%	1 100%
Blue Tongue				
SEV				
1		1 100%	0	0
2		0	1 100%	1 100%
Pale Gums		1 100%	0	0
DAY 23				
No. Observed		1	1	1
Blue Gums				
SEV				
1		0	0	1 100%
Blue Sclera				
SEV				
1		1 100%	1 100%	1 100%
Blue Tongue				
SEV				
1		0	1 100%	0
2		0	0	1 100%
Pale Gums		1 100%	1 100%	0
Pale Tongue		1 100%	0	0

Severity Codes

Observations	Severity No.	Description
Blue Gums/	1	Slight (barely preceptible, slight blue tinged color
Blue Tongue/	2	Moderate (easily seen, blue color)
Blue Sclera	3	Severe (marked, deep blue-purple color)

SUMMARY OF OBSERVATION INCIDENCE

STUDY: 133 SEX: FEMALE

PERIOD	DOSE:(mg/kg) GROUP:	0.5 1-F	0.9 2-F	1.5 3-F
DAY 24				
No. Observed		1	1	1
Blue Gums				
SEV				
1		1 100%	0	1 100%
Blue Sclera				
SEV				
1		1 100%	1 100%	1 100%
Blue Tongue				
SEV				
1		1 100%	0	0
2		0	1 100%	1 100%
Pale Gums		0	1 100%	0
DAY 25				
No. Observed		1	1	1
Blue Gums				
SEV				
2		0	0	1 100%
Blue Sclera				
SEV				
1		1 100%	1 100%	1 100%
Blue Tongue				
SEV				
1		1 100%	0	0
2		0	1 100%	1 100%
Pale Gums		0	1 100%	0

Severity Codes

Observations	Severity No.	Description
Blue Gums/	1	Slight (barely preceptible, slight blue tinged color
Blue Tongue/	2	Moderate (easily seen, blue color)
Blue Sclera	3	Severe (marked, deep blue-purple color)

SUMMARY OF OBSERVATION INCIDENCE

STUDY: 133 SEX: FEMALE

PERIOD	DOSE:(mg/kg) GROUP:	0.5 1-F	0.9 2-F	1.5 3-F
DAY 26				
No. Observed		1	1	1
Blue Gums				
SEV				
1		1 100%	0	1 100%
Blue Sclera				
SEV				
1		1 100%	1 100%	1 100%
Blue Tongue				
SEV				
1		1 100%	0	0
2		0	1 100%	1 100%
Pale Gums		0	1 100%	0
DAY 27				
No. Observed		1	1	1
Blue Gums				
SEV				
1		0	0	1 100%
2		0	1 100%	0
Blue Sclera				
SEV				
1		1 100%	0	1 100%
2		0	1 100%	0
Blue Tongue				
SEV				
1		1 100%	0	0
2		0	1 100%	1 100%
DAY 28				
No. Observed		1	1	1
Scheduled Sacrifice		1 100%	1 100%	1 100%

Severity Codes

Observations	Severity No.	Description
Blue Gums/	1	Slight (barely preceptible, slight blue tinged color
Blue Tongue/	2	Moderate (easily seen, blue color)
Blue Sclera	3	Severe (marked, deep blue-purple color)

APPENDIX 3

Pathology Report

FINAL PATHOLOGY REPORT FOR
FOUR WEEK ORAL DOSE RANGE-FINDING STUDY OF WR242511 IN DOGS
TRL STUDY NUMBER 133

PREPARED
BY
PATHOLOGY ASSOCIATES, INC.
10 WEST 35TH STREET
CHICAGO, IL 60616

FOR
TOXICOLOGY RESEARCH LABORATORY (M/C 868)
DEPARTMENT OF PHARMACOLOGY
UNIVERSITY OF ILLINOIS AT CHICAGO
COLLEGE OF MEDICINE
1940 WEST TAYLOR STREET
CHICAGO, IL 60612-7353

SEPTEMBER 16, 1994

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SECTION I

PATHOLOGY NARRATIVE

FINAL PATHOLOGY REPORT

FOUR WEEK ORAL DOSE RANGE-FINDING STUDY OF WR242511 IN DOGS

INTRODUCTION

This pathology report, submitted by Pathology Associates, Inc. (PAI) to Toxicology Research Laboratory (TRL), University of Illinois at Chicago, represents the pathology findings for the study designated as "Four Week Oral Dose Range-Finding Study of WR242511 in Dogs", Toxicology Research Laboratory Study Number 133.

EXPERIMENTAL DESIGN AND METHODS

Three groups, each composed of two (one male and one female) Beagle dogs, were given the test article (WR242511) at doses of 0.5, 0.9, and 1.5 mg base/kg/day (mbkd) as outlined in the Summary of Experimental Design (Table I) for four weeks. No control group was included. All animals in the study were sacrificed and necropsied at the termination of the study in accordance with TRL Standard Operating Procedures. Tissues required by the protocol for collection at necropsy were preserved in 10% neutral buffered formalin (Table II). No gross lesions were observed at the necropsy.

Tissues required by the protocol to be examined histologically (Table II) were processed and slides were prepared in accordance with PAI Standard Operating Procedures. These tissues were then evaluated by light microscopy, and the results were tabulated.

The pathology portion of the study was conducted in accordance with the protocol, TRL and PAI Standard Operating Procedures, and in the spirit of Good Laboratory Practices (GLP). However, as this was a non-GLP study, no Quality Assurance Statement was issued.

Microscopic findings for all groups are summarized in the Project Summary Tables (Section II). The mean group severity scores are found in the Severity Summary Tables (Section III). The mean group severity scores were determined by dividing the sum of all severity scores for a finding by the number of tissues examined. Microscopic findings in the protocol-required tissues for individual animals are presented in the Tabulated Animal Data Tables (Section IV). The codes used as entries in these tables are explained in the Report Codes Table.

RESULTS AND DISCUSSION

The Results and Discussion section is divided into two parts: Diagnostic Terms and Histopathology Findings. The Diagnostic Terms portion lists and clarifies diagnostic terminology that may be unclear. Terms listed in the Diagnostic Terms portion of this section were not necessarily considered to be test article-related. The Histopathology Findings portion of this section reports the results and provides discussion of the histopathologic evaluation of the tissues.

Diagnostic Terms

The morphologic characteristics of observations and lesions which require comment are presented in subsequent paragraphs to aid in the interpretation of the data.

Lung

Proteinic exudate in the lung consisted of amorphous to fibrillar gray-pink acellular material in the lumen of alveoli. Macrophage infiltrates were found mainly in the interstitial tissues near terminal bronchioles but occurred in other areas of the interstitium and in alveoli. Infiltrating macrophages were large cells with abundant, pale, vacuolated cytoplasm. Some of the larger interstitial macrophage infiltrates also had acute inflammation which consisted of neutrophil infiltration with necrosis. Neutrophils were also found free in some alveoli. Focal chronic inflammation consisted of a single subpleural focus of granulomatous inflammation. Macrophages within this lesion had abundant dense cytoplasm rather than abundant, pale, vacuolated cytoplasm. Lymphocytes were prevalent in this lesion and there was focal, early fibroplasia. No foreign material was seen, but the lesion was typical of a chronic foreign body response. It was considered not to be related to the other lesions described in the lung.

Liver

Swollen hepatocytes were large cells with a ground-glass appearance to the cytoplasm. These cells compressed and obliterated bile canaliculi. Hemosiderin pigment was a golden-brown granular material within Kupffer cell cytoplasm. Cellular infiltrates were focal aggregates of lymphocytes and plasma cells, usually found adjacent to central veins.

Adrenal Gland

Vacuoles in the cytoplasm of cells in the adrenal cortex were variable in size, lacked a delineating membrane, and were clear.

The remainder of the diagnoses used in this study were considered to be self-explanatory and were not discussed in this section.

Histopathology Findings

As there was only one animal per sex in each treatment group and as there was no control group, it was not possible to identify a dose response for any of the changes observed in this study. Changes interpreted as potentially related to the test article were found in the lung, liver, and possibly in the adrenal gland.

Alveolar proteinic exudate, acute alveolar inflammation, and macrophage infiltration occurred in 4 out of the 6 animals in this study (Table III, Summary of Incidence of Potentially Test Article-Related Changes). They did not occur in the low dose female or in the middle dose male. When present, these changes occurred throughout the sections evaluated. However, as sections from only the left apical lobe were evaluated, they may or may not have occurred throughout the entire lung. The absence of these changes from sections evaluated from two animals does not rule out the possibility that they may have occurred in other areas of the lung in these two animals. These changes are consistent with damage to the endothelial or epithelial barriers in the capillary bed of the lung. Similar changes have been associated with oxidative injury following administration of other chemicals. For these reasons and as they occurred in 4 out of 6 animals in this study, alveolar proteinic exudate, acute alveolar inflammation, and macrophage infiltration were considered to be test article-related changes.

Swollen hepatocytes are a common morphologic manifestation of degenerative change in hepatocytes. Swollen hepatocytes occurred in all animals in this study except the low dose male. When present, swollen hepatocytes tended to occur diffusely throughout the sections of liver evaluated. For these reasons, swollen hepatocytes were considered to be a test article-related change. Hemosiderin deposition occurs in normal animals, but can occur as a response to increased erythrocyte turn-over. Hemosiderin deposits occurred in both low dose animals and in one high dose animal in this study. The relationship of the test article to this change is uncertain, though, as hemosiderin deposits were minimal in these dogs and did not occur in one high dose or in either middle dose animal. For these reasons, hemosiderin deposits in the liver were considered to most likely not be a test article-related change. Cellular infiltrates are a common finding in untreated dogs. This change did not occur in either high dose animal and was considered an incidental change.

Vacuolation of cells in the adrenal cortex can be an incidental observation, can be a non-specific finding related to stress, or can be a direct test article-related effect. The occurrence of this change in the middle and high dose females suggests this to be a test article-related finding in this study. This interpretation is uncertain, though, as the vacuolation present in the adrenal cortex was of minimal severity in both affected dogs and could have occurred for several other reasons.

The other lesions observed were considered to be incidental findings and not to warrant further discussion.

CONCLUSIONS

Under the conditions of this study, changes in the lung (proteinic exudates, acute inflammation, and macrophage infiltrates) and liver (swollen hepatocytes) were interpreted as test article-related. Hemosiderin deposits in the liver and vacuolation of cells in the adrenal cortex may have resulted from exposure to the test article or from other causes. Based on the animals affected with these changes and on their severity scores, these two changes were considered to be not related to the test article. A no-effect level was not determined.

Michael J. Tomlinson, DVM, Ph.D.
Diplomate, ACVP

Sept 16, 1994
Date

TABLE I

SUMMARY OF EXPERIMENTAL DESIGN

Treatment Group	Dose Level (mg base/kg/day)	Number of Males	Number of Females
1	0.5	1	1
2	0.9	1	1
3	1.5	1	1

TABLE II

PROTOCOL-REQUIRED TISSUES

* Adrenal glands
Aorta
* Brain (fore-, mid-, and hind-)
Cecum
Colon
Diaphragm
Duodenum
Epididymides
Esophagus
Eyes and optic nerves
Gallbladder
Gross lesions
* Heart
Ileum
Jejunum
* Kidneys
* Liver (with gallbladder drained)
* Lungs/bronchi
Lymph node (submandibular)
Lymph node (mesenteric)
Mammary gland
Muscle (skeletal)
Nerve (sciatic)
* Ovaries
Pancreas
Pituitary
Prostate
Rectum
Rib with marrow
Salivary gland (submandibular)
Skin
Spinal cord (thoracic, cervical)
* Spleen
Stomach
* Testes
Thymus
* Thyroid gland with parathyroids
Tongue
Tonsil
Trachea
Ureter
Urinary bladder
Uterus

* These tissues from all animals were designated for processing and histopathologic evaluation.

TABLE III

SUMMARY OF INCIDENCE OF POTENTIALLY TEST ARTICLE-RELATED CHANGES

DOSE LEVEL mg base/kg/day	0.5		0.9		1.5	
SEX	Male	Female	Male	Female	Male	Female
HISTOPATHOLOGIC CHANGE						
LUNG:						
Exudate, Alveolus, Proteinic	+	-	-	+	+	+
Alveolus, Inflammation, Acute	+	-	-	+	+	+
Infiltrate, Macrophage	+	-	-	+	+	+
LIVER:						
Hepatocyte, Swelling	-	+	+	+	+	+

"+" = present in this animal
"-" = not present in this animal

PATHOLOGY ASSOCIATES, INC.
FOUR WEEK ORAL DOSE RANGE-FINDING STUDY OF WR 242511 IN DOGS
TOXICOLOGY RESEARCH LABORATORY, STUDY NUMBER 133

Report Codes Table

A. Codes applying to organs

N	Tissues within normal histological limits
A	Autolysis precluding adequate evaluation
P	Paired organ missing
U	Tissues unsuitable for complete evaluation
S	Tissues not applicable to animal
*	Tissues not required by protocol

B. Codes applying to microscopic diagnoses

1	minimal
2	mild
3	moderate
4	marked
)	focal
]	locally extensive
>	multifocal
P	Present
B	Neoplasm, benign
M	Neoplasm, malignant without metastasis
C	Neoplasm, malignant with metastasis
X	Metastatic site (+)
-	No data entered

SECTION II

PROJECT SUMMARY TABLE

Project Summary Table

SUMMARY: Incidence of NEOPLASTIC and NON-NEOPLASTIC Microscopic Findings

PROJECT ID. NO: TRL133 FATES: Terminal Sacrifice
WEEKS: 5 SEX: MALE

GROUP:		0.5 mbkd		0.9 mbkd		1.5 mbkd	
NUMBER OF ANIMALS:		1		1		1	
		#	%	#	%	#	%
ADRENAL GLAND	# Ex	1		1		1	
Cortex, hemorrhage		1	(100)	0	(0)	0	(0)
BRAIN (FORE)	# Ex	1		1		1	
BRAIN (MID)	# Ex	1		1		1	
BRAIN (HIND)	# Ex	1		1		1	
HEART	# Ex	1		1		1	
KIDNEY, LEFT	# Ex	1		1		1	
Nephrocalcinosis		1	(100)	1	(100)	1	(100)
KIDNEY, RIGHT	# Ex	1		1		1	
Nephrocalcinosis		1	(100)	1	(100)	1	(100)
LIVER	# Ex	1		1		1	
Hepatocyte, swelling		0	(0)	1	(100)	1	(100)
Infiltrate, cellular		0	(0)	1	(100)	0	(0)
Pigment, hemosiderin		1	(100)	0	(0)	0	(0)
SPLEEN	# Ex	1		1		1	
Capsule, siderofibrotic plaque		1	(100)	0	(0)	0	(0)
THYROID GLAND	# Ex	1		1		1	
PARATHYROID GLAND	# Ex	1		1		1	
Cyst		1	(100)	0	(0)	0	(0)

Project Summary Table

SUMMARY: Incidence of NEOPLASTIC and NON-NEOPLASTIC Microscopic Findings

PROJECT ID. NO: TRL133 FATES: Terminal Sacrifice
WEEKS: 5 SEX: MALE

GROUP:	0.5 mbkd	0.9 mbkd	1.5 mbkd
NUMBER OF ANIMALS:	1	1	1

		#	%	#	%	#	%
TESTIS	# Ex	1		1		1	
LUNG	# Ex	1		1		1	
Alveolus, exudate, proteinic		1	(100)	0	(0)	1	(100)
Alveolus, inflammation, acute		1	(100)	0	(0)	1	(100)
Infiltrate, macrophage		1	(100)	0	(0)	1	(100)
Inflammation, chronic, focal		0	(0)	0	(0)	1	(100)

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Project Summary Table

SUMMARY: Incidence of NEOPLASTIC and NON-NEOPLASTIC Microscopic Findings

PROJECT ID. NO: TRL133 FATES: Terminal Sacrifice
WEEKS: 5 SEX: FEMALE

GROUP:		0.5 mbkd		0.9 mbkd		1.5 mbkd	
NUMBER OF ANIMALS:		1		1		1	
		#	%	#	%	#	%
ADRENAL GLAND	# Ex	1		1		1	
Cortex, vacuolation, cytoplasm		0	(0)	1	(100)	1	(100)
BRAIN (FORE)	# Ex	1		1		1	
BRAIN (MID)	# Ex	1		1		1	
BRAIN (HIND)	# Ex	1		1		1	
HEART	# Ex	1		1		1	
KIDNEY, LEFT	# Ex	1		1		1	
Nephrocalcinosis		1	(100)	1	(100)	0	(0)
KIDNEY, RIGHT	# Ex	1		1		1	
Nephrocalcinosis		1	(100)	1	(100)	0	(0)
LIVER	# Ex	1		1		1	
Hepatocyte, swelling		1	(100)	1	(100)	1	(100)
Infiltrate, cellular		1	(100)	1	(100)	0	(0)
Pigment, hemosiderin		1	(100)	0	(0)	1	(100)
SPLEEN	# Ex	1		1		1	
THYROID GLAND	# Ex	1		1		1	
PARATHYROID GLAND	# Ex	1		1		1	
OVARY	# Ex	1		1		1	

Project Summary Table

SUMMARY: Incidence of NEOPLASTIC and NON-NEOPLASTIC Microscopic Findings

PROJECT ID. NO: TRL133 FATES: Terminal Sacrifice
WEEKS: 5 SEX: FEMALE

GROUP:	0.5 mbkd		0.9 mbkd		1.5 mbkd	
NUMBER OF ANIMALS:	1		1		1	
	#	%	#	%	#	%
LUNG # Ex	1		1		1	
Alveolus, exudate, proteinic	0	(0)	1	(100)	1	(100)
Alveolus, inflammation, acute	0	(0)	1	(100)	1	(100)
Infiltrate, macrophage	0	(0)	1	(100)	1	(100)

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SECTION III

SEVERITY SUMMARY TABLE

Severity Summary Table

PROJECT ID. NO: TRL133 FATES: Terminal Sacrifice
WEEKS: 5 SEX: MALE

GROUP:		0.5 mbkd		0.9 mbkd		1.5 mbkd	
NUMBER OF ANIMALS:		1		1		1	
		#	SEV	#	SEV	#	SEV
ADRENAL GLAND	# Ex	1		1		1	
Cortex, hemorrhage		1	1.00	0		0	
BRAIN (FORE)	# Ex	1		1		1	
BRAIN (MID)	# Ex	1		1		1	
BRAIN (HIND)	# Ex	1		1		1	
HEART	# Ex	1		1		1	
KIDNEY, LEFT	# Ex	1		1		1	
Nephrocalcinosis		1	1.00	1	1.00	1	1.00
KIDNEY, RIGHT	# Ex	1		1		1	
Nephrocalcinosis		1	1.00	1	1.00	1	1.00
LIVER	# Ex	1		1		1	
Hepatocyte, swelling		0		1	1.00	1	1.00
Infiltrate, cellular		0		1	1.00	0	
Pigment, hemosiderin		1	1.00	0		0	
SPLEEN	# Ex	1		1		1	
Capsule, siderofibrotic plaque		1	1.00	0		0	
THYROID GLAND	# Ex	1		1		1	
PARATHYROID GLAND	# Ex	1		1		1	

Severity Summary Table

PROJECT ID. NO: TRL133 FATES: Terminal Sacrifice
WEEKS: 5 SEX: MALE

GROUP:	0.5 mbkd		0.9 mbkd		1.5 mbkd	
NUMBER OF ANIMALS:	1		1		1	
	#	SEV	#	SEV	#	SEV
TESTIS # Ex	1		1		1	
LUNG # Ex	1		1		1	
Alveolus, exudate, proteinic	1	1.00	0		1	1.00
Alveolus, inflammation, acute	1	1.00	0		1	2.00
Infiltrate, macrophage	1	1.00	0		1	2.00
Inflammation, chronic, focal	0		0		1	1.00

* Severity calculated by the number of tissues examined.

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Severity Summary Table

PROJECT ID. NO: TRL133 FATES: Terminal Sacrifice
WEEKS: 5 SEX: FEMALE

GROUP:		0.5 mbkd		0.9 mbkd		1.5 mbkd	
NUMBER OF ANIMALS:		1		1		1	
		#	SEV	#	SEV	#	SEV
ADRENAL GLAND	# Ex	1		1		1	
Cortex, vacuolation, cytoplasm		0		1	1.00	1	1.00
BRAIN (FORE)	# Ex	1		1		1	
BRAIN (MID)	# Ex	1		1		1	
BRAIN (HIND)	# Ex	1		1		1	
HEART	# Ex	1		1		1	
KIDNEY, LEFT	# Ex	1		1		1	
Nephrocalcinosis		1	1.00	1	1.00	0	
KIDNEY, RIGHT	# Ex	1		1		1	
Nephrocalcinosis		1	1.00	1	1.00	0	
LIVER	# Ex	1		1		1	
Hepatocyte, swelling		1	1.00	1	1.00	1	1.00
Infiltrate, cellular		1	1.00	1	1.00	0	
Pigment, hemosiderin		1	1.00	0		1	1.00
SPLEEN	# Ex	1		1		1	
THYROID GLAND	# Ex	1		1		1	
PARATHYROID GLAND	# Ex	1		1		1	
OVARY	# Ex	1		1		1	

Severity Summary Table

PROJECT ID. NO: TRL133 FATES: Terminal Sacrifice
WEEKS: 5 SEX: FEMALE

GROUP:	0.5 mbkd	0.9 mbkd	1.5 mbkd
NUMBER OF ANIMALS:	1	1	1

	#	SEV	#	SEV	#	SEV
LUNG # Ex	1		1		1	
Alveolus, exudate, proteinic	0		1	1.00	1	1.00
Alveolus, inflammation, acute	0		1	1.00	1	2.00
Infiltrate, macrophage	0		1	2.00	1	2.00

* Severity calculated by the number of tissues examined.

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SECTION IV

TABULATED ANIMAL DATA

Tabulated Animal Data

PROJECT ID: TRL133 GROUP: 0.5 mbkd SEX: MALE
WEEKS: 5 FATES: Terminal Sacrifice

ANIMAL ID:	7952
ADRENAL GLAND	
Cortex, hemorrhage	1
BRAIN (FORE)	N
BRAIN (MID)	N
BRAIN (HIND)	N
HEART	N
KIDNEY, LEFT	
Nephrocalcinosis	1
KIDNEY, RIGHT	
Nephrocalcinosis	1
LIVER	
Pigment, hemosiderin	1
SPLEEN	
Capsule, siderofibrotic plaque	1
THYROID GLAND	N

Tabulated Animal Data

PROJECT ID: TRL133 GROUP: 0.5 mbkd SEX: MALE
WEEKS: 5 FATES: Terminal Sacrifice

ANIMAL ID:	7952
PARATHYROID GLAND	
Cyst	P
TESTIS	N
LUNG	
Alveolus, exudate, proteinic	1
Alveolus, inflammation, acute	1
Infiltrate, macrophage	1

Tabulated Animal Data

PROJECT ID: TRL133 GROUP: 0.9 mbkd SEX: MALE
WEEKS: 5 FATES: Terminal Sacrifice

ANIMAL ID:	7951
ADRENAL GLAND	N
BRAIN (FORE)	N
BRAIN (MID)	N
BRAIN (HIND)	N
HEART	N
KIDNEY, LEFT	
Nephrocalcinosis	1
KIDNEY, RIGHT	
Nephrocalcinosis	1
LIVER	
Hepatocyte, swelling	1
Infiltrate, cellular	1
SPLEEN	N
THYROID GLAND	N

Tabulated Animal Data

PROJECT ID: TRL133 GROUP: 0.5 mbkd SEX: FEMALE
WEEKS: 5 FATES: Terminal Sacrifice

ANIMAL ID:	8017
ADRENAL GLAND	N
BRAIN (FORE)	N
BRAIN (MID)	N
BRAIN (HIND)	N
HEART	N
KIDNEY, LEFT	
Nephrocalcinosis	1
KIDNEY, RIGHT	
Nephrocalcinosis	1
LIVER	
Hepatocyte, swelling	1
Infiltrate, cellular	1
Pigment, hemosiderin	1
SPLEEN	N
THYROID GLAND	N

Tabulated Animal Data

PROJECT ID: TRL133 GROUP: 0.5 mbkd SEX: FEMALE
WEEKS: 5 FATES: Terminal Sacrifice

ANIMAL ID:	8017
PARATHYROID GLAND	N
OVARY	N
LUNG	N

Tabulated Animal Data

PROJECT ID: TRL133 GROUP: 0.9 mbkd SEX: FEMALE
WEEKS: 5 FATES: Terminal Sacrifice

ANIMAL ID:	8000
ADRENAL GLAND	
Cortex, vacuolation, cytoplasm	1
BRAIN (FORE)	N
BRAIN (MID)	N
BRAIN (HIND)	N
HEART	N
KIDNEY, LEFT	
Nephrocalcinosis	1
KIDNEY, RIGHT	
Nephrocalcinosis	1
LIVER	
Hepatocyte, swelling	1
Infiltrate, cellular	1
SPLEEN	N
THYROID GLAND	N

Tabulated Animal Data

PROJECT ID: TRL133 GROUP: 0.9 mbkd SEX: FEMALE
WEEKS: 5 FATES: Terminal Sacrifice

ANIMAL ID:	8000
PARATHYROID GLAND	N
OVARY	N
LUNG	
Alveolus, exudate, proteinic	1
Alveolus, inflammation, acute	1
Infiltrate, macrophage	2

Tabulated Animal Data

PROJECT ID: TRL133 GROUP: 1.5 mbkd SEX: FEMALE
WEEKS: 5 FATES: Terminal Sacrifice

ANIMAL ID:	7999
ADRENAL GLAND	
Cortex, vacuolation, cytoplasm	1
BRAIN (FORE)	N
BRAIN (MID)	N
BRAIN (HIND)	N
HEART	N
KIDNEY, LEFT	N
KIDNEY, RIGHT	N
LIVER	
Hepatocyte, swelling	1
Pigment, hemosiderin	1
SPLEEN	N
THYROID GLAND	N
PARATHYROID GLAND	N

Tabulated Animal Data

PROJECT ID: TRL133 GROUP: 1.5 mbkd SEX: FEMALE
WEEKS: 5 FATES: Terminal Sacrifice

ANIMAL ID:	7999
OVARY	N
LUNG	
Alveolus, exudate, proteinic	1
Alveolus, inflammation, acute	2
Infiltrate, macrophage	2

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APPENDIX 4

Protocol and Protocol Amendments

Contract No.: DAMD17-92-C-2001
Task Order No.: UIC-7I
UIC/TRL Study No.: 133

FOUR WEEK ORAL DOSE RANGE-FINDING STUDY OF WR242511 IN DOGS

1.0 PURPOSE OF THE STUDY:

The purpose of this study is to determine the toxicity of WR242511 tartrate in dogs following four weeks of daily administration by gelatin capsule. Results derived from this study will be used to determine dose levels for the "Four Week Oral Toxicity Study of WR242511 in Dogs". The protocol for this study was approved by the UIC Animal Care Committee (Appendix 1).

2.0 SPONSOR:

2.1	Name:	U.S. Army Medical Materiel Development Activity
2.2	Address:	Fort Detrick Frederick, MD 21702-5009
2.3	Representative:	George J. Schieferstein, Ph.D.

3.0 TESTING FACILITY:

3.1	Name:	Toxicology Research Laboratory (TRL)
3.2	Address:	University of Illinois at Chicago (UIC) Department of Pharmacology 1940 W. Taylor St. Chicago, Illinois 60612-7353
3.3	Study Director:	Barry S. Levine, D.Sc., D.A.B.T.

4.0 DATES:

4.1	Proposed Initiation of Dosing:	02/09/94
4.2	Proposed End of In-Life Phase:	03/09/94
4.3	Proposed Study Completion Date (Draft Study Report):	05/06/94

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5.0 TEST ARTICLE

5.1 Name or Code No: WR242511 Tartrate
Bottle Number will be indicated in the raw data.

5.2 TRL Chemical No: 1720614

5.3 Physical Description: Yellow powder

5.4 Stability and Handling of Test Article:

5.4.1 Temperature: -20 to -15°C.

5.4.2 Humidity: Ambient conditions at -20 to -15°C.

5.4.3 Light: Protect from light.

5.4.4 Special Requirements: None.

5.5 Special Handling Procedures: Standard safety precautions will be followed including gloves, eye protection, mask, and lab coats.

5.6 Log of Test Article: The amount, date, identity of person(s) removing aliquots and the purpose for which each aliquot of the test article was removed from the batch will be documented. At termination of the study, all unused test article will be returned to the Sponsor.

6.0 PERSONNEL:

Study Director	Barry S. Levine, D.Sc., D.A.B.T.
Toxicologist	Clyde W. Wheeler, Ph.D.
Pathologist	Michael J. Tomlinson, D.V.M., Ph.D., D.A.C.V.P.
Pathology Support	Ralph M. Bunte, D.V.M., D.A.C.V.P.
Analytical Chemist	Adam Negrusz, Ph.D.
Clinical Veterinarian	Terry Hewett, D.V.M., D.A.V.C.P.
Veterinarian Support	Documented in raw data
Tox. Lab Supervisor	Soudabeh Soura, B.S.
Lead Technician	Documented in raw data
Chemistry Specialist	Thomas Tolhurst, B.S.
Clinical Pathology	Maria Lang, A.H.T., C.V.T.

7.0 TEST SYSTEM:

7.1 Species: Dog

7.2 Strain: Beagle

7.3 Number and Sex: 3 Males and 3 Females

7.4 Age of Animals: Approximately 7 - 9 months old at dosing initiation.

7.5 Weight of Animals: Approximately 10 - 13 kg (males) and approximately 8 - 11 kg (females) at dosing initiation.

7.6 Source of Animals: Marshall Farms, North Rose, NY.

7.7 Justification for Selection of Test System: The FDA requires the use of two animal species, one being a non-rodent, in preclinical toxicology studies. The dog is a standard and accepted non-rodent species for regulatory toxicology studies, and is specified by the Sponsor.

7.8 Procedure for Unique Identification of Test System: Upon arrival each animal will be given a facility unique number. This number will appear as an ear tattoo and will also appear on a cage card visible on the front of each run. The cage card will additionally contain the study number, test article identification, treatment group number and dose level. Cage cards will be color-coded as a function of treatment group. Raw data records and specimens will also be identified by the unique test animal number.

7.9 Housing: The animals will be housed in an AAALAC- accredited facility. Animals will be housed singly per run in a temperature (65 - 84°F) and humidity (50 $\pm$ 20%) controlled room with a 12 hour light/12 hour dark cycle. The run size, at least 15 feet^2, is adequate to house dogs at the upper weight range as described in the *Guide for the Care and Use of Laboratory Animals*, DHHS (NIH) No. 86.23. All runs will be cleaned and fresh bedding replaced daily. The runs will be sanitized once every two weeks.

7.10 Quarantine Procedure: Animals will be quarantined for at least two weeks. During that time, the animals will be observed daily for signs of illness and all unusual observations will be reported to the Study Director, Toxicologist, or Clinical Veterinarian. Body weights and physical examinations will be done upon the dogs' arrival at the animal facility. Additionally, each dog will be lightly sprayed upon arrival with PARA PYRETHRIN MIST for fleas, lice, and ticks. Within one week of arrival, hematology and clinical chemistry tests, and fecal examination for internal parasites will be performed. If parasites are found, the affected animal will be treated with a vermifuge approved by the Sponsor, and at least 10 days and a negative fecal examination will elapse before the animal is used on a study. All dogs will have been vaccinated against canine distemper, infectious canine hepatitis, leptospirosis, parainfluenza, parvo,

oral papilloma, and rabies by the animal supplier. Animals will be examined during quarantine and approved for use by the Clinical Veterinarian prior to being placed on test. Any sickly animal will be eliminated from the animal selection process. If a selected animal appears sickly prior to initiation of treatment, it will be replaced by a healthy animal prior to treatment under the direction of the Study Director or Toxicologist. Quarantine release will be documented on the Clinical Veterinarian Log by the veterinarian prior to study initiation.

7.11 Food: Purina Certified Canine Diet No. 5007 (Ralston Purina Company, St. Louis, MO), approximately 400 g, will be provided daily from arrival until termination. Exactly 400g will be provided when food consumption is measured. The food will be removed for an overnight fast (≈ 16 - 20 hours) prior to blood collection or scheduled sacrifice.

7.12 Water: Tap water from an automatic watering system in which the room distribution lines are flushed daily will be provided *ad libitum* from arrival until termination. The water is untreated with additional chlorine or HCl.

7.13 There are no known contaminants in the feed or water which are expected to influence the study. The results of bi-monthly comprehensive chemical analyses of Chicago water are documented in files maintained by Quality Assurance.

7.14 It is not known if the animals will experience pain or distress during the study. Analgesic or anesthetic agents will confound the ability to determine the toxic potential of the test article, and therefore will not be used. If an animal is in severe pain or distress, following consultation with the veterinary staff, it will be euthanized in accordance with standard operating procedures.

8.0 EXPERIMENTAL DESIGN:

8.1 Treatment Groups:

Treatment Group	Dose Level (mg base/kg/day)	Number of Males	Number of Females
1	0.5	1	1
2	0.9	1	1
3	1.5	1	1

WR242511 dose levels will be selected on the basis of a previously conducted two week oral dose range-finding study in rats (UIC/TRL Study No. 106), following consultation with the Sponsor. The number of animals, 1 sex/dose, is the minimum number of dogs and is the number typically used in preliminary dose range-finding studies.

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If toxicity is not observed after two weeks of treatment, the mid dose may be escalated above the high dose for the remainder of the treatment period.

8.2 Frequency and Route of Administration of the Test Article: The test article will be administered once daily by gelatin capsule starting with Day 0 for at least four weeks. All animals will receive empty gelatin capsules for the last 3 days during Week -1 to acclimate them to the procedure. The quantity of the test article (mg base/kg) will be adjusted based on the animals most recent body weight. The animals will be dosed up to and including the day prior to scheduled necropsy on Day 28.

8.3 Justification of Route: The oral route is the intended clinical route and is specified by the Sponsor.

8.4 Procedure to Control Bias during the Assignment of Animals to Treatment Groups:During the quarantine/pretest period, the animals will be randomized by sex using a table of random letters or numbers. The method will be documented in the raw data.

8.5 Test Article Vehicle: Gelatin capsules (size 000; capacity 1.37 ml).

8.6 Test Article Dosage Form Preparation and Analyses: Not applicable.

8.7 Type and Frequency of Observations, Tests, Analyses and Measurements:

8.7.1 Clinical Signs: All animals will be observed once daily for clinical signs of toxicity approximately 1 - 2 hours after dosing. Additionally, all animals will be observed for morbidity/mortality in the afternoon and immediately prior to dosing in the morning.

8.7.2 Clinical Observations: All animals will be subjected to a physical examination including examination of eyes and all orifices at randomization (Week -1), on Day 0 (initiation of dosing), weekly thereafter, and at termination on Day 28.

8.7.3 Body Weight: Body weights of all animals will be recorded at randomization in Week -1, weekly thereafter, and at termination on Day 28.

8.7.4 Food Consumption: Food consumption for all animals will be measured over an approximate 24 hour period weekly commencing with Week -1.

8.7.5 Clinical Pathology: Hematology and clinical chemistry parameters will be measured following an overnight fast approximately one week prior to dosing initiation, on Day 14 and on Day 28 at termination. In addition, methemoglobin levels will be measured weekly commencing on Day 0, just prior to dosing. On Day 21, in addition to the measurement of methemoglobin levels prior to dosing, non-fasted levels will be determined approximately 2 and 8 hours after treatment. The animals will be unanesthetized and sufficient blood will be collected from the jugular vein to measure the following parameters in random order. Water will be available *ad libitum* during all fasting periods.

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Hematology

Activated partial thromboplastin time	Mean corpuscular hemoglobin (MCH)
Erythrocyte count	Mean corpuscular hemoglobin concentration (MCHC)
Erythrocyte morphology	Mean corpuscular volume (MCV)
Heinz bodies	[a]Methemoglobin
Hematocrit	Platelet count
Hemoglobin	Prothrombin time
Leukocyte count, total and differential	Reticulocyte count

[a] To be measured with a Co-oximeter (Instrumentation Laboratory, Model No. 282). The assay will be performed within one-hour of sample collection. The specimens will be kept on wet ice prior to analysis.

Clinical Chemistry

Alanine aminotransferase (ALT/SGPT)	Globulin (calculated)
Albumin	Glucose
Albumin/globulin ratio (calculated)	Haptoglobin
Alkaline phosphatase	Lactate dehydrogenase (LDH)
Aspartate aminotransferase (AST/SGOT)	Phosphorus (inorganic)
Calcium	Potassium
Chloride	Sodium
Cholesterol	Total bilirubin
Creatinine	Total protein
Creatine kinase (CK)	Triglycerides
Gamma glutamyl transferase	Urea nitrogen (BUN)

8.7.6 Plasma and Blood Cell Isolation: Just prior to dosing, a minimum of 2.5 ml of blood will be collected from the jugular vein weekly commencing on Day 0 for the separation and isolation of plasma and cellular blood components according to the Sponsor's directives. The plasma and cell fractions resulting from separation by centrifugation will be sent to Col. Thomas Brewer, MD as specified by the Sponsor. The results obtained from these samples will not be included in the study report.

8.7.7 Pathology: All animals which die on test or are killed if moribund will be necropsied. All remaining animals which survive the four week test period will be sacrificed and necropsied on Day 28. This will be accomplished by sodium pentobarbital anesthesia (i.v.; 20-30 mg/kg) and exsanguination. An extensive necropsy will be performed under the direction and supervision of the pathologist. Terminal body weights will be collected prior to routine sacrifice.

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The necropsy procedure will be a thorough and systematic examination and dissection of the animal viscera and carcass to include the external surface, all orifices, the cranial cavity, external surface of the brain, cross section of the spinal cord, the nasal cavity and nasal turbinates, thoracic, abdominal and pelvic cavities and their viscera, and cervical tissues and organs. The following tissues and organs will be collected and fixed in 10% neutral buffered formalin (NBF).

*Adrenal glands
Aorta
*Brain (fore-, mid-, and hind-)
Cecum
Colon
Diaphragm
Duodenum
Epididymides
Esophagus
Eyes and optic nerve
Gall bladder
Gross lesions
*Heart
Ileum
Jejunum
*Kidneys
*Liver (with gall bladder drained)
*Lungs/Bronchi
Lymph node (submandibular)
Lymph node (mesenteric)
Mammary gland
Muscle (skeletal)
Nerve (sciatic)
*Ovaries
Pancreas
Pituitary
Prostate
Rectum
Rib with marrow
Salivary gland (submandibular)
Skin
Spinal cord (thoracic, cervical)
*Spleen
Stomach
*Testes
Thymus
*Thyroid gland with parathyroids
Tongue
Tonsil
Trachea
Ureter
Urinary bladder
Uterus

Those tissues marked with an asterisk (*) in all treatment groups found dead, sacrificed either *in extremis* or at scheduled necropsy on Day 28 will be embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically.

In addition to the collection of the aforementioned tissues and organs, five tubes of heparinized blood (≈ 250 ml) will be collected at euthanasia and bile will be aspirated by syringe from the gall bladder at necropsy according to the Sponsor's directives. The samples will be sent to Col. Thomas G. Brewer, MD as specified by the Sponsor, and the results obtained from these samples will not be included in the study report.

8.7.8 Statistical Analyses: Statistical analyses will not be conducted due to the small sample size. Quantitative data will be tabulated and presented in the report. In addition to the written report, summary data tables of parameters and variability will

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be transmitted to the Sponsor on magnetic media (computer diskette) in "ASCII" form. The transcribed data on disk will no longer be considered to be GLP compliant.

9.0 RECORDS TO BE MAINTAINED:

All data generated during the conduct of the study, except those that are generated as direct computer input, shall be recorded directly, promptly, and accurately in ink in bound books with prenumbered pages or on worksheets that shall be bound during or at the conclusion of the nonclinical laboratory study. All appropriate computer and machine output shall be bound during or at the conclusion of the study. All data entries shall be dated on the day of entry and signed or initialed by the person entering the data.

Any changes in entries for whatever reason (e.g., to correct an error or transposition) shall be made so as not to obscure the original entry, shall indicate the reason for such change, and shall be dated and signed or identified at the time of data input. In computer driven collection systems, the operator responsible for direct data input shall be identified at the time of data input. Any changes in computer entries for whatever reason (e.g., to correct an error or transposition) shall be made in such a manner so as not to obscure the original entry, if possible, shall indicate the reason for such change, and shall be dated and the responsible individual shall be identified.

All recorded data shall be reviewed, signed, and dated by a knowledgeable person, other than the person making the entry, to assure adherence to procedures and to verify observations.

Upon completion of the study and submission of the final report, all raw data, documentation, specimens, test article reserves and other materials necessary to reconstruct the study will be stored in the TRL archives maintained by Quality Assurance.

All changes or revisions, and reasons therefore, to this protocol once it is approved shall be documented, signed by the Study Director and Sponsor, dated and maintained with the protocol.

10.0 REGULATORY REQUIREMENTS:

This study will be performed within the spirit of the UIC/TRL Quality Assurance Program designed to conform with FDA Good Laboratory Practice Regulations and EPA Good Laboratory Practice Standards.

Will this study be submitted to a regulatory agency? Yes If so, to which agency(ies)? Food and Drug Administration
Does the Sponsor Request that test article samples be returned? Possibly; direction to be provided by Sponsor.
Does the Sponsor request that samples of the test article/carrier mixture(s) be returned to the Sponsor? Not applicable

UIC The University of Illinois at Chicago

Office of the Vice Chancellor for Research (M/C 672)
310 Administrative Office Building
1737 West Polk Street
Chicago, Illinois 60612-7227
(312) 996-4995

Appendix 1

November 22,1993

Barry S. Levine
Med-Pharmacology
312 BGRC, M/C 868

Dear Dr. Levine:

The protocol indicated below has been reviewed in accordance with the Animal Care Policies of the University of Illinois at Chicago and approved on May 18, 1993.

Title of Application: **Four Week Oral Dose Range-Finding Study of WR242511 In Dogs**

ACC Number: 93-033-12

This institution has Animal Welfare Assurance Number A3460.01 on file with the Office for Protection from Research Risks, NIH. Please transmit this letter of acceptable verification of your research protocol to your sponsor.

Thank you for complying with the Animal Care Policies and Procedures of UIC.

Sincerely yours,

Josephine B. Miller

Josephine B. Miller, Ph.D.
Chair, Animal Care Committee

JBM:st
xc:BRL

11.0 PROTOCOL APPROVAL:

STUDY DIRECTOR: Barry S. Levine, D.Sc., D.A.B.T. — Date: 11/19/93

SPONSOR APPROVAL: George J. Schieferstein, Ph.D. Contracting Officer's Representative (COR) — Date: 12/13/93.

COMMENTS FROM THE COR:

PROTOCOL AMENDMENT

Study No.: 133

Title: Four Week Oral Dose Range-Finding Study of WR242511 in Dogs

1. Page 2 Section 5.1

Indicate the Bottle Number of the test article; "BM05816".

Reason: Sponsor requested that specific bottle number be included in the protocol.

2. Page 4 Section 7

Add the following section:

"7.14 It is not known if the animals will experience pain or distress during the study. Analgesic or anesthetic agents will confound the ability to determine the toxic potential of the test article, and therefore will not be used. If an animal is in severe pain or distress, following consultation with the veterinary staff, it will be euthanized in accordance with standard operating procedures."

Reason: Sponsor requested addition to the protocol.

3. Page 4 Section 8.1

Add the following sentence to the end of the first paragraph "The number of animals, 1 sex/dose, is the minimum number of dogs and is the number typically used in preliminary dose range-finding studies."

Reason: Sponsor requested addition to the protocol.

4. Page 5 Section 8.5

Change the test article vehicle section to the following "Gelatin capsules (size 000; capacity 1.37 ml)."

Reason: Clarification of the size and capacity of the gelatin capsules to be used.

5. Page 6 Section 8.7.6

Change first sentence to indicate that all blood collection will be done "just prior to dosing" and that the plasma and cellular components will be separated according to the Sponsor's directives.

PROTOCOL AMENDMENT

Study No.: 133

Title: Four Week Oral Dose Range-Finding Study of WR242511 in Dogs

5. contd.

Reason: Clarification of the time when blood collection will be performed and that the separation will be performed according to the SOP provided by the Sponsor.

6. Page 6 Section 8.7.7

Add "(i.v.; 20-30 mg/kg)" after "sodium pentobarbital anesthesia".

Reason: Clarification of the protocol to indicate the dose and route of phenobarbital.

7. Page 7 Section 8.7.7

A) Change scheduled necropsy date from "Day 14" to "Day 28" in the second paragraph.

B) Change third paragraph to indicate that "≈ 250 ml" of heparinized blood will be collected at euthanasia and bile at necropsy according to the Sponsor's directives in an SOP to be provided by the Sponsor.

Reason: Mistake in protocol (A) and Sponsor requested change in the protocol (B).

Approvals:

STUDY DIRECTOR: Barry S. Levine, D.Sc. D.A.B.T. 12/10/93 Date

SPONSOR APPROVAL: George J. Schieferstein, Ph.D. Contracting Officer's Representative (COR) 12/13/93 Date

PROTOCOL AMENDMENT

Study No.: 133

Title: Four Week Oral Dose Range-Finding Study of WR242511 in Dogs

8. Page 1 Section 4.0

Add the study dates as follows:

4.1	Proposed Initiation of Dosing:	02/09/94
4.2	Proposed End of In-Life Phase:	03/09/94
4.3	Proposed Study Completion Date (Draft Study Report):	05/06/94

Reason: The study dates have been finalized.

9. Page 4 Section 8.1

Change the dose levels to read as follows:

"Low" = "0.5" mg base/kg/day

"Mid" = "0.9" mg base/kg/day

"High" = "1.5" mg base/kg/day

Reason: Dose levels have been selected following consultation with the Sponsor.

10. Page 5 Section 8.7.5

Change the first sentence to indicate clinical pathology parameters will be measured "approximately one week prior to dosing initiation" in place of "within one week of arrival".

Reason: Clarification of the protocol.

Approvals:

STUDY DIRECTOR: Barry S. Levine
Barry S. Levine, D.Sc. D.A.B.T. 2/11/94
Date

SPONSOR APPROVAL: G Schieferstein
George J. Schieferstein, Ph.D. 3/18/94
Contracting Officer's Representative (COR) Date

PROTOCOL AMENDMENT

Study No.: 133

Title: Four Week Oral Dose Range-Finding Study of WR242511 in Dogs

11. Page 5 Section 8.7.5

Add the following sentence "On Day 21, in addition to the measurement of methemoglobin levels prior to dosing, non-fasted levels will be determined approximately 2 and 8 hours after treatment."

Reason: Sponsor requested addition to the study.

12. Page 7 Section 8.7.7

Add "Lungs/Bronchi" to the list of tissues which will be embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically.

Reason: The lungs were a potential target organ in a previously conducted rat toxicity study.

Approvals:

STUDY DIRECTOR: _____ 7/7/94
Barry S. Levine, D.Sc. D.A.B.T. Date

SPONSOR APPROVAL: _____ 9/9/94
George J. Schieferstein, Ph.D. Date
Contracting Officer's
Representative (COR)

APPENDIX 5

Study Deviations

Contract No.: DAMD17-92-C-2001
Task Order No.: UIC-7I
UIC/TRL Study No.: 133

FOUR WEEK ORAL DOSE RANGE-FINDING STUDY OF WR242511 IN DOGS

Study Deviation*

Deviation Type	Specific Deviation	Effect on Study
Protocol	On several occasions, the relative humidity in the animal room deviated outside the specified range by ≤ -7%.	None. These sporadic occurrences were not considered to have had an impact on the outcome of the study.

*The detailed "Deviation Report" is contained in the raw data which is archived at the University of Illinois at Chicago, Department of Pharmacology, Chicago, Illinois.

The above deviation did not affect the integrity of the study.

Barry S. Levine, D.Sc., D.A.B.T.

9/22/94
Date